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PESTICIDES PROGRAM
National Communicable
Disease Center
Atlanta, Ga. 30333

Cardiovascular Effects of o,p'-DDD

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Daily oral administration of o,p'-DDD to dogs produced glucocorticoid deficiency. This was demonstrated by a decreased plasma hydroxy corticosteriods (17 OHCS) and the abolition of ACTH-induced increase in 17 OHCS and decrease of eosinophiles. Plasma volume, Na+, K+, and blood pressure and heart rate were unaltered. After two weeks of treatment with o,p'-DDD the dogs were anesthetized for pharmacological evaluation. Blood pressure and heart contractile force and rate responses to graded doses of epinephrine and norepinephrine were determined in treated and in untreated dogs. As a final test, cardiac contractile force response to ouabain was determined.

Vasopressor and cardio-accelerator responses to ouabain were similar in treated and untreated dogs. However, the positive inotropic effect of the amines was depressed in treated dogs. The o,p'-DDD-induced glucocorticoid deficiency produced little apparent direct circulatory effect but diminished the animals' ability to withstand stressful procedures, such as thoracotomy and multiple injections of vasopressor amines. Blood pressure and plasma volume declined progressively in treated but not in normal dogs. These effects in DDD-treated dogs were prevented by the administration of Prednisolone for three days prior to surgery.

La administración oral de o,p'-DDD a los perros produce deficiencia de los glucocorticoides. Se describe como fué demostrada esta observación. La presión sanguínea y la fuerza contráctil cardiaca como respuesta a dosis de epinefrina y norepinefrina fueron determinadas en perros que habián recibido el agente y otro grupo que no lo había recibido. En una prueba final la fuerza de contractilidad cardíaca como respuesta a la ouabaina fué determinada

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The Determination of Pentachlorophenol and Hexachlorophene in Human Adipose Tissue

by

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The identification and quantitation of potential chemical pollutants and their metabolites in human adipose tissue are of great importance for monitoring human exposure to these environmental compounds. The two highly chlorinated phenols, pentachlorophenol (PCP) and hexachlorophene [2,2-methylenebis (3,4 6-trichlorophenol] (HCP), are widely used, and it will be of great interest to establish the extent of their storage in human adipose tissue.

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The toxicological importance of PCP has been described by BEVENUE and BECKMAN (1967) and that of HCP by GUMP (1969), KIMBROUGH (1971) and KIMBROUGH and GAINES (1971). Adequate methods of analysis in urine and several tissues where relatively high levels of these compounds are present by virtue of acute high exposure have been developed, and urinary excretion rates have been established.

Gas chromatographic analysis of PCP in human urine and blood after formation of the alkyl ethers was reported by CRANMER and FREAL (1970), BEVENUE, et al (1968), and RIVERS (1971). BARTHEL et al (1969) described a method for the determination of PCP in blood, urine, tissue and clothing in which the underivatized PCP was injected on a gas chromatographic column containing 3% diethylene glycol succinate (DEGS) plus 2% syrupy phosphoric acid.

Electron capture gas chromatography was used to determine HCP in urine, blood, tissues and agricultural samples after methylation to produce the corresponding dimethoxy ether [2,2' methylenebis (3,4,6-trichloromethoxybenzene)] (CURLEY and HAWK, 1971, and GUTENMANN and LISK, 1970). PORCARO and SHUBIAK (1968) PORCARO et al. (1969) determined HCP on human skin and blood by gas chromatography after preparing the silyl ether. BACHMANN and SHETLAR (1969) determined HCP in biological tissue and fluids by electron capture gas chromatography without preparing a derivative. The purpose of this investigation was to develop a method

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for the determination of low levels of PCP and HCP in human adipose tissue of the general population.

EXPERIMENTAL

Gas chromatograph, Micro-Tek MT-220 equipped with tritium foil electron capture detector, and two U-shaped glass columns: a) For HCP: 2' x 1/4" o.d., packed with 60-80 mesh Gas Chrom Q coated with 2% SE-30; nitrogen flow rate 100 ml/min. b) For PCP: 6' x 1/4" o.d., packed with 80/100 mesh Chromosorb W. HP coated with 4% SE-30 + 6% QF-1; nitrogen flow rate 60 ml/min. Temperatures: Inlet, 230°C; columns, 200°C; detector, 215°C; transfer line, 240°C.

Tissue grinder, Duall Kontes #885380; Chromaflex column, size 22, Kontes #420100; Centrifuge tubes, 13-ml glass stoppered and graduated; Concentrator tubes, 10-ml. Kontes 570050; Disposable pipets; centrifuge.

Reagents

Sodium hydroxide AR; Potassium hydroxide AR; Hydrochloric acid AR; diethyl ether AR; Benzene and hexane nanograde; N-ethyl-N'-nitro-N-nitrosoguanidine, Aldrich Chemical Co.; Sodium sulfate anhydrous extracted with benzene in a Soxhlet extractor; Silica gel, Woelm, activity grade I; distilled water extracted with benzene.

Preparation of Diazoethane Reagent

Prepare the reagent using the method of STANLEY (1966). The reagent should be prepared daily and stored in a freezer. The adherence to the manufacturer's instructions for handling the reagent is recommended.

Preparation of Silica Gel Microcolumn:

Dry the silica gel for 48 hours at 170°C and store in an oven maintained at 130°C. On the day of use, remove the adsorbent from the 130°C oven to a desiccator and allow to come to room temperature. Weigh 10 grams of the dry silica gel into a vial with a Teflon-lined screw cap, add 0.10 ml water and immediately cover the container tightly and mix. Allow the adsorbent to equilibrate for 2-3 hours with intermittent mixing. No particles should adhere to the sides of the vial after equilibration. The standardized silica gel will contain approximately 1% water.

Just prior to use, prepare the chromatographic columns by adding 1 gram of silica gel (1% water) to the column which has been plugged with a small, loose wad of glass wool. Add 2 g. of anhydrous sodium sulfate. Introduce the derivatized PCP and HCP in hexane onto the column.

Preparation of Standard Solutions:

a) PCP: Prepare hexane solutions at concentrations of 2,4, and 20 ng/0.1 ml.

b) HCP: Prepare hexane solutions at concentrations of 2,20 and 200 ng/0.1 ml.

Preparation of Standard Curves:

Prepare mixtures of PCP and HCP as listed below by pipetting 0.1 ml aliquots of the appropriate individual standard solutions into 13-ml centrifuge tubes.

1) 2 ng PCP + 2 ng HCP

2) 4 ng PCP + 20 ng HCP

3) 20 ng PCP + 200 ng HCP

To each tube add 1.5 ml hexane and 0.5 ml 10% NaOH solution. Mix on a Vortex mixer for 1 min. and centrifuge to separate the two layers. Aspirate the hexane layer and discard. Add another 1.5 ml hexane, mix, centrifuge and discard the hexane layer. Repeat the hexane extraction once more. Add 0.5 ml concentrated HCl to the aqueous solution, mix and allow to cool to room temperature. Add 1.5 ml diethyl ether, mix on a Vortex mixer for 30 seconds and allow the two layers to separate. Transfer the ether layer to a 13-ml centrifuge tube with a disposable pipet. Repeat the ether extraction twice, transferring each ether extract to the 13-ml centrifuge tube. In a well ventillated hood, add 2 ml of freshly prepared dizaoethane and allow to stand for 20 minutes. Remove excess reagent by bubbling dry nitrogen through the solution, using a disposable pipet for delivery. Continue bubbling nitrogen until the solution has evaporated. Add 1 ml hexane and 0.5 g anhydrous sodium sulfate, and mix for a few seconds on the Vortex mixer. Using the nitrogen delivery pipet, transfer the hexane extract containing ethylated PCP and HCP to the silica gel microcolumn.

Rinse the centrifuge tube with 1 ml hexane and transfer to the column. Repeat the hexane rinse and transfer to the column once more. Allow the hexane to sink into the column and discard that eluate. Add 8 ml 10% benzene in hexane to the column and collect this fraction, which contains ethylated PCP. As soon as the solvent sinks in the column, change collection tubes and continue eluting with 8 ml 60% benzene in hexane. This fraction contains ethylated HCP.

Adjust the volume of the first fraction (10% benzene in hexane) if necessary to exactly 8 ml with hexane. Inject 10 µl from each of the three concentrations of PCP into the gas chromatograph. The sensitivity of the electron capture detector to PCP derivative is approximately 2.5 pg.

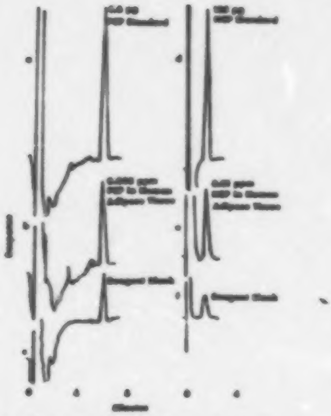
Concentrate the second fraction (60% benzene in hexane) to a volume of 0.5 ml under a gentle stream of nitrogen. Inject 10 µl from each of the three concentrations of HCP into the gas chromatograph. The sensitivity of the electron capture detector to ethylated HCP is 40 pg. A complete reagent blank must be run through the entire procedure with each set of samples and standards.

Analysis of Adipose Tissue:

Accurately weigh approximately 200 mg of adipose tissue directly into a Duall tissue grinder. Add 1 ml of hexane, mix on Vortex mixer, and allow to stand for about 30 min. Grind the sample, remove the pestle and wash it with 0.5 ml hexane, allowing the hexane wash to drain into the grinding tube. Add 0.5 ml 10% NaOH, proceed with analysis as described in the preparation of standard curves beginning with "Mix on Vortex mixer for 1 min...."

RESULTS AND DISCUSSION

The ethylated derivatives of PCP and HCP were selected over the methylated because of their better gas chromatographic properties. The limits of detectability for PCP and HCP in adipose tissue are 5 and 10 ppb, respectively. Typical chromatograms of PCP and HCP in human fat at the reported levels of detectability are shown in Figure 1. The background from the reagent blank did not allow the detection of lower levels of PCP and HCP.



To establish recovery of both PCP and HCP from human adipose tissue, a pooled sample was prepared by rendering adipose tissue from several individuals at 60°C in a water bath for about two hours. The melt was filtered through glass wool to remove any connective or muscle tissues. The sample of pooled and rendered fat was prepared in order to eliminate sample variations. The sample was kept in a freezer to minimize possible loss or deterioration.

Samples from the pooled human fat weighing 100-300 mg were analyzed for both PCP and HCP using the procedure described in the analysis of adipose tissue. An average of 5 ppb PCP was found in six replicates and 30 ppb HCP in 10 replicates of human adipose tissue. Replicate samples of the pooled fat (100-300 mg) were spiked with 4.6 ng PCP and 10 ng HCP. The average recoveries of PCP and HCP from the replicates analyzed were 75% and 96%, respectively.

Table 1 illustrates the levels of PCP and HCP in human adipose tissue samples from the general population. The HCP content ranged from N.D. to 80 ppb with a mean value of 20 ppb, if the high HCP content of sample 3 is not included.

TABLE 1

Sample No.	PCP	HCP	Sample No.	PCP	HCP
1	19	20	11	22	ND
2	17	30	12	14	30
3	52	1000	13	35	20
4	45	30	14	52	ND
5	23	20	15	24	ND
6	25	20	16	12	10
7	21	10	17	12	10
8	35	ND	18	29	ND
9	17	10	19*	5	30
10	20	80			

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* Pooled and rendered human fat sample.
ND = Not Detectable.

It can be concluded on the basis of the analysis of human adipose tissue samples that humans are continuously exposed to low levels of PCP and HCP from the environment, food supplies, and disinfectants. The possibility of toxicological effects from continued exposure of animals and humans to PCP and HCP at high and low levels has been considered, but to date there are no available studies to establish the "no effect" level of these toxicants. The procedure described in this paper is being made available to the pharmacologists and toxicologists to aid them in their studies.

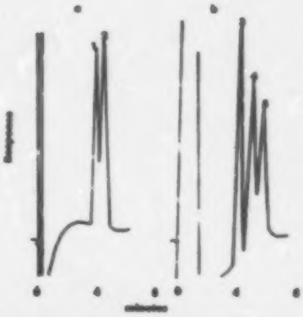

Fig. [illegible] Chromatograms of Various Derivatives of [illegible]
a G.C. column [illegible] Nitrogen flow rate [illegible] ml/min, oven temp. [illegible]°C, [illegible] detector
1 - methyl derivative
2 - ethyl derivative
b G.C. column [illegible] Nitrogen flow rate [illegible] ml/min., oven temp. [illegible]°C [illegible] detector
3 - ethyl derivative
4 - trimethylsilyl derivative
5 - chloroacetate derivative

In addition to the sensitivity of the method for the determination of low levels of PCP and HCP in fat, it has an inherent advantage in its ability to provide confirmation of identity. The use of aqueous sodium hydroxide and the extraction of this basic solution with hexane to remove intact chlorinated hydrocarbons which interfere with the determination confirms that the compounds remaining in the alkali media are acidic compounds which form sodium salts upon treatment with a base. The preparation of other derivatives of PCP and HCP can also be used as further confirmation of identity. CRANMER and FREAL (1970) prepared the methyl, ethyl, propyl, isobutyl, butyl, isoamyl, amyl ethers of PCP, which have different retentions when injected onto various gas chromatographic columns. The methyl derivative (CURLEY and HAWK, 1971), ethyl and silyl derivatives (PORCARO and SHUBIAK, 1968, PORCARO et al, 1969) and chloroacetate derivative (SHAFIK et al, 1971) of HCP are also separated by gas chromatography. Figure 2 illustrates chromatograms of various derivatives of HCP.

Silica gel column chromatography employing selective differential elution of PCP with 10% benzene in hexane and HCP with 60% benzene in hexane is a further means of confirmation.

Confirmation of the diethyl ether of hexachlorophene and the ethyl ether of pentachlorophenol was accomplished by mass spectral direct probe analysis.

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A Cleanup Procedure for the Determination of Low Levels of Alkyl Phosphates, Thiophosphates, and Dithiophosphates in Rat and Human Urine

Mohammed T. Shafik,* Diane Bradway, and Henry F. Enos

A method has been developed for the cleanup of rat and human urine extracts which permits quantitative analysis of alkyl phosphates at a level considerably lower than heretofore possible. The method has been used for analysis of urine from rats exposed to parathion. Results indicate that the analysis of urine for alkyl phosphates constitutes an excellent tool for monitoring both acute and chronic exposure to parathion in the general population.

An investigation has been initiated to determine whether a correlation exists between the extent of exposure to an organophosphorus pesticide and the urinary secretion of the corresponding alkyl phosphate. As part of the study, Shafik and Enos (1969) developed a method for the determination of alkyl phosphates in the urine of humans occupationally exposed to organophosphorus pesticides. However, application of this method to the determination of these compounds in rat urine required a preliminary cleanup. Although several authors (Askew *et al.*, 1969; Gutenmann *et al.*, 1968) have described analytical methods for determining alkyl phosphates in a variety of substrates, no cleanup procedure has been suggested. This study was therefore undertaken to develop a suitable sample cleanup.

The preparation of standard curves, extraction of urine, and gas chromatographic determination of the methylated and ethylated alkyl phosphates have been described in a previous publication (Shafik and Enos, 1969). *O,O*-Diethyl phosphorothionate is quantitated after methylation as *O,O*-diethyl *S*-methylphosphorothiolate (Shafik *et al.*, 1970).

The initial phase of this investigation involved the separation of mixtures of trialkyl phosphate standards by solvent partitioning and column chromatography.

For the convenience of the reader, the following list includes the abbreviations used when referring to alkyl phosphates: DMP—*O,O*-dimethyl phosphate; TMP—*O,O,O*-trimethyl phosphate; DMEP—*O,O*-dimethyl *O*-ethyl phosphate; DEP—*O,O*-diethyl phosphate; DEMP—*O,O*-diethyl *O*-methyl phosphate; TEP—*O,O,O*-triethyl phosphate; DMTP—*O,O*-dimethyl phosphorothionate; TMTP—*O,O,O*-trimethyl phosphorothionate; DMETP—*O,O*-dimethyl *O*-ethyl phosphorothionate; DETP—*O,O*-diethyl phosphorothionate; DEMTP—*O,O*-diethyl *O*-methyl phosphorothionate; TETP—*O,O,O*-triethyl phosphorothionate; DEPTh—*O,O*-diethyl phosphorothiolate; DEMPTh—*O,O*-diethyl *S*-methyl phosphorothiolate; TEPTh—*O,O*-diethyl *S*-ethyl phosphorothiolate; DMDTP—*O,O*-dimethyl phosphorodithioate; DMEDTP—*O,O*-dimethyl *S*-ethyl phosphorodithioate; TMDTP—*O,O*-dimethyl *S*-methyl phosphorodithioate; DEDTP—*O,O*-diethyl phosphorodithioate; DEMDTP—*O,O*-diethyl *S*-methyl phosphorodithioate; TEDTP—*O,O*-diethyl *S*-ethyl phosphorodithioate.

MATERIALS AND METHODS

Solvents and Reagents. Silica gel, Woelm, Activity Grade I (Waters Associates, Inc.), was activated at 135° C for 48 hr and stored in a desiccator. Standard solution for determining silica gel elution pattern was prepared as a mixture of the trialkyl phosphates in hexane at the following concentrations: TMTP, 5 μg/ml; TETP, 5 μg/ml; TMP, 20 μg/ml; DMEP, 19 μg/ml; DEMP, 12 μg/ml; TEP, 12 μg/ml; TMDTP, 25 μg/ml; TEDTP, 10 μg/ml; and TEPTh, 10 μg/ml.

Animal Treatments. Pairs of male Sprague-Dawley rats (250–270 g body weight) were dosed by gavage with peanut oil solutions of parathion at 160, 16, 3.2, and 1.6 μg/ml, representing, respectively, $^{1}/_{10}$, $^{1}/_{100}$, $^{1}/_{500}$, and $^{1}/_{1000}$ of the LD$_{50}$ of parathion (LD$_{50}$, 4 mg/kg), and an aqueous solution of the potassium salt of diethyl phosphorothioate at a level equivalent to the DETPK content of $^{1}/_{10}$ of the LD$_{50}$ of parathion (114 μg/ml). The doses were administered daily for 12 days. The animals were maintained in stainless steel metabolism cages (Acme Metal Products Inc.) which were cleaned twice daily to minimize fecal contamination. Urine samples were collected in glass bottles, with no preservative added, at 24-hr intervals before, during, and for 12 days after exposure. Twenty-four-hour urine samples from rats fed parathion were analyzed for alkyl phosphates at day 0, 1, 6, 12, and 14. For the DETPK feeding, an extra 24-hr sample was analyzed at day 2.

Solvent Partitioning of Trialkyl Phosphates. Evaporate the methylated or ethylated dialkyl phosphate standard solution to 0.5 ml using a gentle stream of filtered nitrogen and a water

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Table I. Typical Elution Pattern of Trialkyl Phosphates from Silica Gel. Recovery % of Indicated Amount[a] of Test Compound

Solvent	TMTP, 8 μg	TETP, 5 μg	TMP, 20 μg	DMEP, 19 μg	DEMP, 12 μg	TEP, 12 μg	TMDTP, 25 μg	TEDTP, 10 μg	TEPTh, 10 μg
Hexane	...	...	...	...	...	...	...	...	...
20% (v/v) benzene in hexane	...	...	...	...	...	...	...	...	...
40% (v/v) benzene in hexane	...	...	...	...	...	...	13	51	...
60% (v/v) benzene in hexane	100	100	...	...	...	...	87	49	...
80% (v/v) benzene in hexane	...	...	...	...	...	...	...	...	...
Benzene	...	...	...	...	...	...	...	...	...
20% (v/v) ethylacetate in benzene	...	...	...	...	...	...	...	...	84
40% (v/v) ethylacetate in benzene	...	...	...	2	17	46	...	...	16
60% (v/v) ethylacetate in benzene	...	...	19	57	71	51	...	...	...
80% (v/v) ethylacetate in benzene	...	...	66	40	12	2	...	...	...
Ethylacetate	...	...	15	...	...	...	...	...	...

[a] The original amount of trialkyl phosphates in 1 ml of mixed standard.

Table II. Recovery of Known Amounts of Trialkyl Phosphates Added to Human and Rat Urine Extracts Using the Cleanup Procedure. Concentration[a] of Indicated Compound, ppm

Trialkyl phosphate	Human urine		Rat urine	
	Added	Recovered	Added	Recovered
TMTP	0.10	0.092	0.50	0.42
	0.010	0.009	0.050	0.046
TETP	0.10	0.093	0.50	0.44
	0.010	0.009	0.050	0.044
TMDTP	0.50	0.49	2.50	2.40
	0.050	0.046	0.25	0.24
TEDTP	0.20	0.19	1.0	0.91
	0.020	0.016	0.10	0.080
TEPTh	0.20	0.18	1.0	0.94
	0.020	0.016	0.10	0.10
DMEP	0.40	0.22	2.0	1.57
	0.040	0.027	0.20	0.17
DEMP	0.20	0.17	1.0	0.79
	0.020	0.019	0.10	0.10

[a] All results calculated as the potassium salt of the corresponding dialkyl phosphate.

bath maintained at 40° C. Add 9.5 ml of water and 10 ml of *n*-hexane, mix on a Vortex mixer for 1 min, and allow the layers to separate. The hexane will extract the sulfur-containing trialkyl phosphates: methylated and ethylated DMTP, DMDTP, DETP, DEPTh, and DEDTP. The nonsulfur trialkyl phosphates will remain in the aqueous layer. Add 5 g of NaCl and 10 ml of benzene to the aqueous layer. Mix on a Vortex mixer for 1 min and allow the layers to separate. The benzene extract contains DMEP, DEMP, TEP, and a portion of the TMP.

Elution of Trialkyl Phosphates from Silica Gel. Partially deactivate the silica gel by shaking with 3% (w/w) distilled water. Allow to stand for about 4 hr. To a size 22 chromatographic column (Kontes #420100) plugged with glass wool, add 1 g of silica gel, followed by 2 g of anhydrous sodium sulfate. Prewash the column with 10 ml of *n*-hexane and discard. Introduce 1 ml of the solution of trialkyl phosphates to the silica gel column. Elute successively with 8 ml of each of the solvents listed in Table I, collecting each eluate separately.

Cleanup Procedure for Trialkyl Phosphates in Rat and Human Urine. A combination of the benzene partitioning step and the silica gel column chromatography was applied to the cleanup of both rat and human urine using the following procedure. Concentrate the methylated or ethylated ether–acetonitrile extract of urine (Shafik and Enos, 1969) to 0.5 ml in a water bath maintained at 40° C, using a gentle stream of nitrogen. Add 9.5 ml of water, 5 ml of benzene, and 5 g of NaCl. Mix on a Vortex mixer for 1 min and allow the layers to separate. With the aid of a disposable pipet, transfer the benzene extract quantitatively to a silica gel column prepared as previously described. Immediately begin collection of the eluate. Extract the aqueous layer with an additional 3 ml of benzene, transferring the benzene extract to the column just as the previous extract reaches the sodium sulfate layer. Rinse the sides of the column with 2 ml of benzene. The benzene eluate collected up to this point will contain the methylated and ethylated thionates and dithioates. Change receivers and continue elution of the column with 8 ml of 40% ethyl acetate in benzene. This fraction contains methylated and ethylated diethyl phosphorothiolate. A third fraction, eluted with 8 ml of 80% ethyl acetate in benzene, contains methylated and ethylated DEP and ethylated DMP. Most of the TMP is retained on the silica gel column. Eluates are quantitated by gas chromatography (MicroTek MT 220), using a dual flame photometric detector operating in the phosphorus and sulfur modes.

Rat and human urine extracts to which known quantities of trialkyl phosphates had been added are analyzed by this method to obtain recovery data.

RESULTS AND DISCUSSION

Chromatograms representing standard mixtures of trialkyl phosphates were previously presented (Shafik and Enos, 1969; Shafik *et al.*, 1970). It has been established that quantitation should be based on ethylated DMP and DMTP and methylated DEP, DMDTP, and DEDTP; quantitation of DETP should be based on the methylated thiolate isomer.

Table I shows the order of elution of a mixture of trialkyl phosphate standards from silica gel. Although both the methyl and ethyl derivatives of each sulfur-containing dialkyl phosphate were available, only one derivative of each compound was included in the standard mixture. Preliminary studies showed that the methyl and ethyl derivative elute in the same fraction.

The recoveries of known amounts of trialkyl phosphates from rat and human urine extracts are shown in Table II.

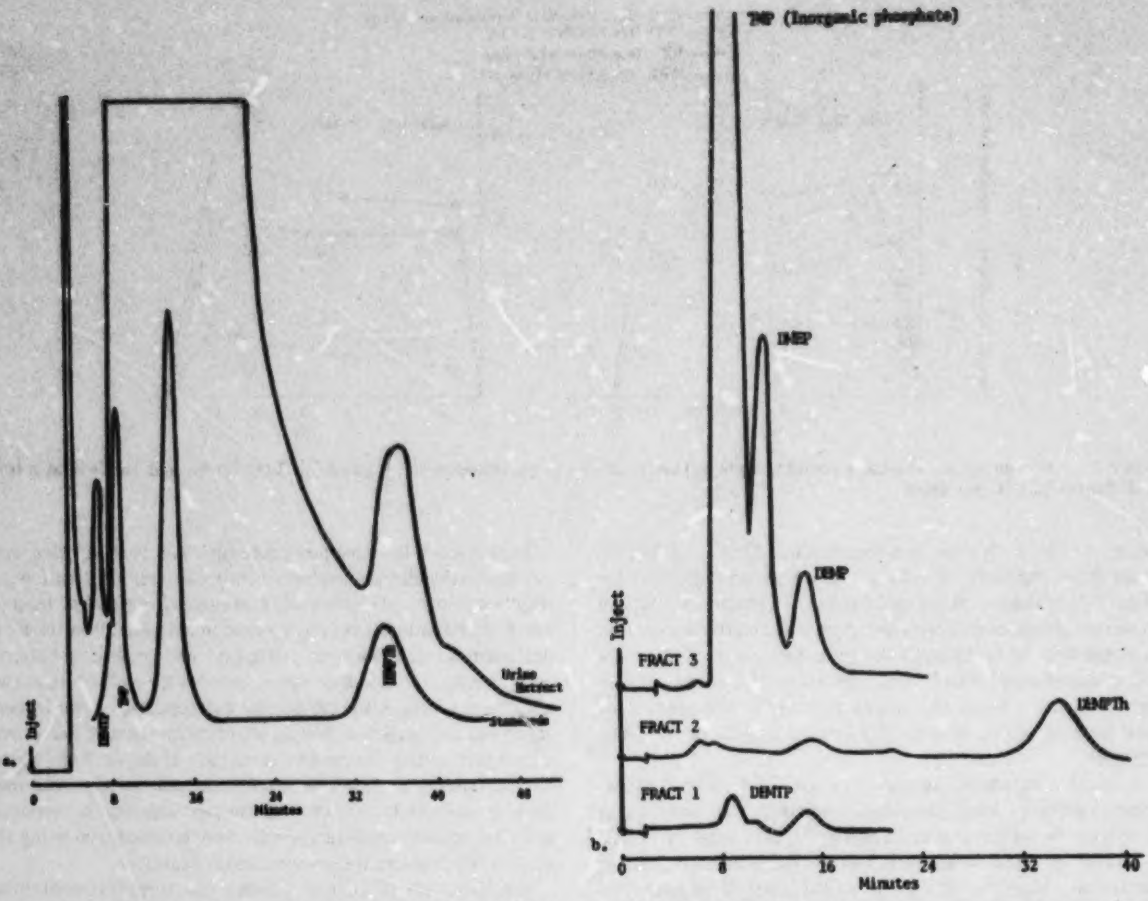

Figure 1. a. Chromatograms of standard alkyl phosphates and noncleaned-up methylated urine extract from rats exposed to $^1/_{100}$ LD$_{50}$ parathion. Extract equivalent to 0.1 ml rat urine injected. Phosphorus mode, 526 mμ. b. Chromatograms of the three fractions from cleanup of methylated rat urine extract following exposure to $^1/_{100}$ LD$_{50}$ parathion. Each injection represents 0.1 ml rat urine

Because of the instability of aqueous standard solutions of the potassium salts of dialkyl phosphates, these salts were not added directly to urine. The trialkyl phosphates were added to the alkylated urine extracts. Previous work (Shafik and Enos, 1969) has established that dialkyl phosphates are quantitatively extracted from acidified urine with acetonitrile–ether. Two spiking levels were used for each compound. As in the study of the elution pattern, only one of the two derivatives of each dialkyl phosphate was used. Trimethyl phosphate and triethyl phosphate were not included, since these compounds could also result from the alkylation of inorganic phosphate. Recoveries from human urine extracts spiked at levels of 0.01 to 0.50 ppm are generally greater than 80%. Recoveries from rat urine extracts spiked at levels of 0.05 to 2.5 ppm are in the range of 80 to 100%.

Table III shows limits of detectability of trialkyl phosphates in rat and human urine with and without the cleanup. Data are given only for the derivatives used for actual quantitation of alkyl phosphates in urine, *i.e.*, methylated DEP, DMDTP, DEDTP, and DEPTh and ethylated DMP and DMTP. These data clearly indicate the value of cleanup of urine extracts for gas chromatographic analysis. It is possible to realize at least a fivefold increase in sensitivity for most trialkyl phosphates. Before cleanup injections of urine extracts could not exceed the equivalent of 0.02 ml of rat urine and 0.1 ml of human urine and still permit quantitation of all six trialkyl phosphates. The addition of a cleanup step permits the injection of urine extracts equivalent to 0.1 and 0.5 ml of rat and human urine, respectively, as shown in Figures 1a and 1b.

Table III. The Limits of Detectability of Trialkyl Phosphates in Rat and Human Urine Before and After Cleanup. Lower Limits[a] of Detection, ppm

Trialkyl phosphate	Human urine		Rat urine	
	Cleanup	No cleanup	Cleanup	No cleanup
DMETP	0.001	0.01	0.007	0.1
TMDTP	0.1	0.7	0.1	2
DEMDTP	0.002	0.01	0.01	0.1
DEMTP[b]	0.003	0.01	0.01	0.2
DMEP	0.002	0.01	0.01	0.1
DEMP	0.001	0.01	0.006	0.5

[a] All results calculated as the potassium salt of the corresponding dialkyl phosphate. [b] Calculated as DEMPTh.

Application of the cleanup procedure to urine of rats exposed to parathion gave satisfactory quantitative results at the two higher levels of exposure, $^1/_{10}$ of the LD$_{50}$ and $^1/_{100}$ of the LD$_{50}$. However, cleanup was not sufficient to permit quantitation of alkyl phosphates at the two lower levels. The results of the exposure experiment are presented in

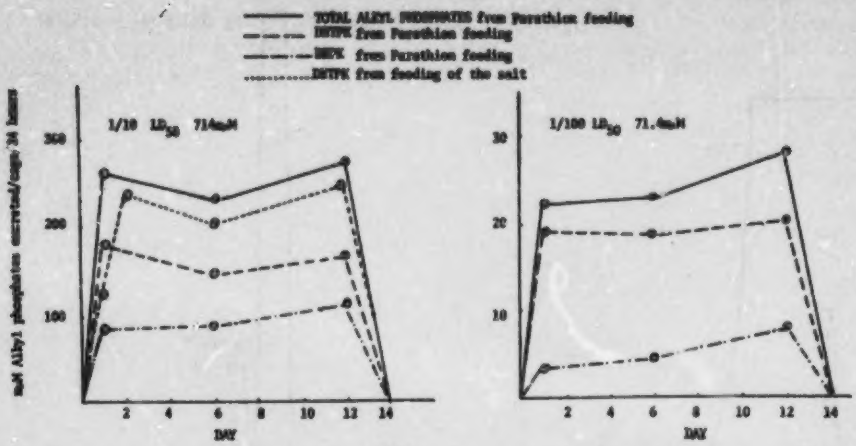

Figure 2. Alkyl phosphate excretion resulting from exposure of rats to parathion at the $^1/_{10}$ and $^1/_{100}$ LD_{50} levels, and DETPK at a level equivalent to $^1/_{10}$ LD_{50} parathion

Figure 2. In each case two metabolites, DEP and DETP, result from exposure of rats to parathion and account for 30 to 40% of the parathion initially fed. The total amount of these two compounds appeared to remain relatively constant from the first 24 hr through the remaining days of exposure and dropped rapidly to 0 upon cessation of feeding. There appears to be a small but steady increase in the amount of DEP formed, which is especially evident at $^1/_{100}$ of the LD_{50} exposure.

A linear relationship appears to exist between level of exposure and total alkyl phosphate excretion. In some cases there may be an advantage in using the sum total of DETP and DEP excreted in the urine as an index of exposure to parathion. However, from a practical point of view, it may be preferable to determine exposure from DETP alone since coextracted inorganic phosphate interferes to some extent with the quantitation of low levels (0.01 ppm) of DEP.

The results of the experiment in which DETPK was fed to rats are also included in Figure 2. The levels and excretion pattern of DETP are very similar to those of total alkyl phosphates (DEP + DETP) following exposure to an equivalent amount of parathion. It is interesting to note that no oxidation of the potassium salt of DETP occurs in the rat, as evidenced by the absence of DEP in the urine. This suggests that the DEP which is found following exposure to parathion arises from the action of hydrolytic enzyme(s) on paraoxon, a metabolite of parathion.

Table IV. Cholinesterase Levels in Rats Following Exposure to Parathion and DETPK

Exposure level	Cholinesterase, μm/ml/min Plasma	Red blood cell
$^1/_{10}$ LD_{50} parathion	1.5	1.6
	2.5	2.5
$^1/_{100}$ LD_{50} parathion	4.4	3.3
	4.4	2.9
$^1/_{500}$ LD_{50} parathion	4.0	2.7
	4.4	2.6
$^1/_{1000}$ LD_{5} parathion	3.6	3.4
	...	2.3
68 μg DETPK	2.3	2.5
	2.9	3.3
Control	4.6	3.6
	4.4	3.4

In this work the cleanup procedure has been applied specifically to the alkyl phosphate metabolites resulting from parathion exposure. However, it is an extremely versatile method and has intentionally been presented in detail to allow its use in determining exposure to virtually all organophosphorus compounds. Depending upon the compound of exposure and the resulting alkyl phosphate metabolites, or the level of exposure, an analyst is free to select those steps of the procedure which satisfy his particular needs. If the dialkyl thioates or dithioates are of specific interest, hexane partitioning may provide sufficient cleanup. If further cleanup is required, silica gel column chromatography may be employed using the solvent which elutes the compound(s) of interest.

Similarly, when interest centers on nonsulfur-containing alkyl phosphates, the hexane preextract may be discarded and the compounds of interest isolated with benzene partitioning. For further cleanup the benzene extract may be introduced into a silica gel column and eluted with a suitable prior solvent to remove less polar interfering compounds, followed by elution with 80% ethyl acetate in benzene to recover the alkyl phosphate. An analyst may use his experience to decide whether any steps may be added or eliminated and which fractions may be combined or discarded.

An additional advantage inherent in the method is its ability to separate the alkyl phosphate esters by group and thus provide additional confirmation of identity. Solvent partitioning separates sulfur-containing compounds from those which do not contain sulfur. The elution pattern from silica gel provides even more characteristic separation and confirmation.

In addition to cleanup of urine extracts and confirmation of the identity of trialkyl phosphates, the procedure may be used for the preparation of pure standards, the separation of thionates from thiolates, and the isolation of sufficient quantities of the compounds for mass spectroscopy or other confirmatory procedures.

Since depression of blood cholinesterase has long been considered an index of exposure to organophosphorus insecticides, these values were obtained for comparison to urinary alkyl phosphate excretion. Blood taken from each rat approximately 2 hr following administration of the final doses of parathion and DETPK and from control rats was analyzed for plasma and red blood cell cholinesterase activity (Cranmer and Peoples, 1970). Table IV lists the plasma and red blood cell cholinesterase levels for each rat. Cholinesterase inhibition is evident following exposure to $^{1}/_{10}$ of the LD$_{50}$ of parathion but at lower levels of exposure no inhibition was observed. In comparison alkyl phosphates are easily detectable at a level of exposure of $^{1}/_{100}$ of the LD$_{50}$, which is equivalent to 40 μg of parathion per kilogram of body weight.

An unexpected result of this experiment was the apparent inhibition of cholinesterase by the potassium salt of DETP. This same inhibition was observed in the subsequent exposure of two additional pairs of rats to DETPK. Analysis of the potassium salt revealed no gross impurities which might account for these observations. Further studies are being conducted on this problem.

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The authors thank Morris F. Cranmer and Anita Peoples for performing the cholinesterase determinations.

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CHOLINESTERASE RESPONSE AND SYMPTOMATOLOGY FROM EXPOSURE TO ORGANIC PHOSPHORUS INSECTICIDES

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WENATCHEE, WASH., is the center of extensive orchards. These are dependent on irrigation, and for topographical reasons only a limited amount of land can be irrigated. These and other factors place an unusual premium on the maximum production per acre of high-quality fruit. To obtain this production it has long been necessary for orchardists in the area to use pesticides extensively.

The introduction of the organic phosphorus insecticides brought new problems. During the 1950 season two young children died in Wenatchee, and although their unrelated deaths were ascribed to unknown causes, poisoning was suspected. Poisoning was suspected also in the death of one man and in the illnesses of a young woman and of two children. The Public Health Service, at the request of the State Health Department, cooperated in an investigation. No final conclusion regarding the cause of death of the children or of the man could be reached. It was shown that, with the possible exception of the man, the persons who were ill in any degree either had no known exposure or their exposure had been minimal. However, it was not known at the time whether minimal exposure could lead to death. This and other unanswered questions suggested the need of a more extensive investigation.

Since this study was begun, other similar work has been reported. The most closely related in subject matter is the paper by Kay and his collaborators[1] who studied orchardists using organic phosphorus insecticides in Quebec. The same authors presented a useful review of the literature regarding the effects of agricultural and industrial exposure.

MATERIALS AND METHODS

The present report covers studies which were carried out during the year 1951. A total of 258 persons were studied, and of these 246 contributed one or more blood samples. Eight

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From the Communicable Disease Center, Public Health Service, United States Department of Health, Education, and Welfare, Atlanta.

Pharmacologist (Dr. Sumerford); Senior Surgeon (Dr. Hayes); Senior Assistant Surgeon (R) (Dr. Johnston); Chemist (Mr. Walker), and Junior Assistant Sanitarian (R) (Miss Spillane).

[1] Kay, K.; Monkman, L.; Windish, J. P.; Doherty, T.; Pare, J., and Racicot, C.: Parathion Exposure and Cholinesterase Response of Quebec Apple Growers, A. M. A. Arch. Indust. Hyg. 6:252, 1952.

hundred five samples of red blood cells and 802 samples of plasma were analysed for their cholinesterase content. Determinations were made by the electrometric method of Michel.[2]

The persons studied were divided into eight groups. Three of these groups, mixing-plant personnel (MPP), commercial applicators (CA), and part-time applicators (PTA), had extensive occupational exposure. Two other groups, workers in orchards (WO) and the group composed of fieldmen, warehousemen, and miscellaneous workers (FWM), were known to have definite, though often minimal or irregular, occupational exposure. The sixth group was constituted of residents living near orchards (RNO) who had no known occupational connection with sprays or dusts containing organic phosphorus insecticides. Residents within 500 ft. of an orchard were said to be "near," but actually most of the subjects in this group lived in orchards with trees on two or three sides of the house. The seventh group included residents in the Wenatchee area living far from orchards (RFO) so that they had no demonstrated contact with insecticides; yet, the possibility of a minimal exposure could not be excluded. Finally, the eighth group included persons living completely outside the fruit-growing area (ROA); it was concluded after appropriate questioning that these persons had no opportunity for exposure.

Of the 258 persons studied, 223 were studied in sufficient detail to have their symptomatology considered in this report. The subjects were questioned in regard to a list of 110 symptoms arranged as a systems review. The list, though limited, was selected to cover illnesses in general and was purposely not restricted in any way to the disorders known to be associated with organic phosphorus insecticides. Usually the health of the person was investigated, and a blood sample was taken at the same time for cholinesterase determination.

Persons working in the apple industry in the Wenatchee area have greater exposure to parathion[3] than to tetraethylpyrophosphate, especially during the early part of the spray season.[4] Even so, it has not been possible in many case histories to distinguish exposure to parathion from exposure to tetraethylpyrophosphate, and no effort is made in the report to separate the effects of these two organic phosphorus insecticides except in special instances.

By the same token, it has not been possible to distinguish between the effects of sprays and dust. Sprays were generally made up from water-wettable powder, and those who prepared the sprays received their most concentrated exposure from this powder. Furthermore, mixing-plant personnel and the pilots and aircraft loaders among the commercial applicators were exposed to a higher proportion of dust than the other groups, but it was not possible to determine whether the greater effects which they generally showed were caused by their greater exposure to dust or merely by their greater exposure to different formulations.

RESULTS

Cholinesterase Values.—Table 1 summarizes all the observations made on cholinesterase values during 1951. The Table is divided into four portions, representing the preexposure, exposure, recovery, and postrecovery periods during which blood samples were taken. This distinction between periods was made individually for each sample and was based on the best information which could be obtained regarding the exposure to insecticides of the person who contributed the sample.

The preexposure values given in the Table represent within the limits of the experiment "normal" values for different occupational groups. Each sample on which these values are based was taken before the individual person had any known exposure to an organic phosphorus insecticide during the year 1951. Furthermore, since the samples were taken almost entirely during the late spring just before the spray season, the persons from whom they were taken had had a maximum period for recovery from any exposure which they might have experienced during the year 1950. Samples from persons outside the area showed an average red cell cholinesterase value of 0.72 and an average plasma cholinesterase value of 0.91. This may be compared with a range of 0.67 to 0.86, reported as average red cell values for the Michel method by different authors, and a range of 0.70 to 0.97, reported as average plasma values by the same authors in various published and unpublished reports. The results of these different authors have been summarized and analyzed in detail by Wolfsie and Winter.[5]

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[2] Michel, H. O.: An Electrometric Method for the Determination of Red Blood Cell and Plasma Cholinesterase Activity, J. Lab. & Clin. Med. 34:1564, 1949.

[3] O,O-diethyl O-*p*-nitrophenyl thiophosphate.

[4] Batchelor, G. S.: Survey of Insecticide Spray Practices Used in the Fruit Orchards of North Central Washington, A. M. A. Arch. Indust. Hyg., this issue, p. 399.

TABLE 1.—*Cholinesterase Values of Different Occupational and Check Groups as Related to Exposure to Organic Phosphorus Insecticides*

Exposure Period	Occupational Group	No. of Persons	No. of Samples	Cholinesterase Values (Δ pH/Hr.)					
				Red Blood Cells			Plasma		
				Range	Mean	Std. Error	Range	Mean	Std. Error
Preexposure	MPP	2	2	0.73 to 0.81	0.82	0.09	0.89 to 1.03	0.96	0.07
	CA	12	12	0.70 to 0.94	0.79	0.02	0.70 to 1.07	0.88	0.04
	PTA	22	23	0.59 to 0.96	0.78	0.02	0.47 to 1.25	0.95	0.04
	WO	21	21	0.44 to 0.95	0.76	0.03	0.61 to 1.28	0.87	0.04
	FWM	5	5	0.59 to 0.88	0.75	0.05	0.69 to 1.05	0.89	0.07
	RNO	34	35	0.52 to 1.00	0.75	0.05	0.47 to 1.45	0.89	0.03
	RFO	..	..	…………	….	….	…………	….	….
	ROA	16	28	0.61 to 0.79	0.72	0.02	0.59 to 1.25	0.91	0.04
Exposure	MPP	9	30	0.11 to 0.86	0.46	0.05	0.10 to 0.89	0.39	0.07
	CA	47	158	0.15 to 0.94	0.66	0.01	0.17 to 1.17	0.68	0.02
	PTA	63	170	0.09 to 0.97	0.60	0.02	0.06 to 1.25	0.70	0.01
	WO	17	78	0.40 to 0.89	0.67	0.01	0.51 to 1.19	0.81	0.02
	FWM	16	31	0.66 to 0.91	0.74	0.01	0.46 to 1.08	0.73	0.03
	RNO	43	60	0.16 to 1.12	0.70	0.02	0.12 to 1.09	0.57	0.03
	RFO	3	3	0.69 to 0.74	0.72	0.01	0.63 to 0.81	0.80	0.06
Recovery	MPP	5	6	0.49 to 0.78	0.65	0.04	0.62 to 0.89	0.77	0.05
	CA	21	25	0.26 to 0.89	0.64	0.03	0.51 to 1.19	0.86	0.03
	PTA	15	33	0.15 to 0.86	0.62	0.03	0.17 to 0.99	0.60	0.03
	WO	8	9	0.60 to 0.84	0.71	0.03	0.66 to 1.30	0.89	0.06
	FWM	2	2	0.64 to 0.81	0.72	0.09	0.58 to 0.88	0.73	0.09
	RNO	4	4	0.60 to 1.02	0.77	0.09	0.74 to 1.05	1.06	0.10
	RFO	9	16	0.63 to 0.85	0.74	0.02	0.64 to 1.01	0.83	0.03
Postrecovery	MPP	4	4*	0.62 to 0.88	0.75	0.05	0.54 to 0.94	0.78	0.12
	CA	18	31	0.47 to 0.74	0.66	0.02	0.72 to 1.14	0.91	0.05
	PTA	7	14	0.56 to 0.86	0.69	0.02	0.49 to 0.99	0.78	0.13
	WO	1	2	0.59 to 0.63	0.61	0.02	1.15 to 1.19	1.17	0.01
	FWM	4	5	0.67 to 0.86	0.75	0.03	0.65 to 1.13	0.84	0.09
	RNO	3	3	0.51 to 0.73	0.60	0.07	0.72 to 0.88	0.79	0.07
	RFO	2	2	0.69 to 0.73	0.71	0.02	0.87 to 1.06	0.97	0.10

* Only three plasma values were available.

The over-all average for all the preexposure values taken during 1951 in Wenatchee was 0.77 for the red blood cells and 0.88 for the plasma. The lowest average value observed within any group in Wenatchee was 0.75 for the red cell cholinesterase and 0.87 for the plasma cholinesterase. These values fall within the range of means reported for normal, completely unexposed persons.

It is true that a few low values were observed in samples taken during the preexposure period. This fact is reflected in the ranges presented in Table 1. One value for red cell cholinesterase and two for plasma fell below 0.50. In the first instance, the red cell sample was the only one taken from the person during the year; the corresponding plasma value (1.03) was entirely normal. In the two instances involving low plasma values, one case was of a person who contributed only a single blood sample during the season; the red cell value was also reduced (0.52). The second case in which a very low plasma value was shown during the preexposure period was of an entirely different sort. The subject contributed two blood samples in May, one in June, and one in September. In each instance the erythrocyte cholinesterase value (0.85 to 0.93) was normal and slightly above the average; the plasma values (0.47 to 0.55) were consistently low. No attempt can be made to explain the three low cholinesterase values just mentioned. However, it appears likely that an explanation should be sought in the situation surrounding each individual sample rather than in the preconceived idea that persons in the Wenatchee area failed to recover normal cholinesterase levels from one season to another.

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[5] Wolfsie, J. H., and Winter, G. D.: Statistical Analysis of Normal Human Red Blood Cell and Plasma Cholinesterase Activity Values, A. M. A. Arch. Indust. Hyg. 6:43, 1952.

Examination of that portion of Table 1 which deals with exposure indicates that at least some persons in all three groups known to have definite and consistent exposure to organic phosphorus insecticides showed marked depletion of their cholinesterase values during the spray season. The exposure of mixing-plant personnel was more nearly uniform than the exposure of other groups and was so great that the average exposure period values were 0.46 for red blood cell cholinesterase and 0.39 for plasma cholinesterase. These average values, it will be seen, are about equal to or lower than the extreme low values for the total population observed during the preexposure period. The average cholinesterase values for commercial applicators and part-time applicators showed smaller but significant depletion. The values for fieldmen, warehousemen, and miscellaneous workers and for other workers in orchards showed a depletion which was so small that its significance cannot be evaluated at this time. The average for residents living near orchards showed a very slight depletion due entirely to low values for two cases. These cases involved two children who, through gross carelessness, were allowed to play with bags from which water-wettable parathion powder had been partially emptied. One of the boys became violently ill but recovered; the other, presumably less exposed, did not become sick. Their cases have been described elsewhere in detail.[6] When these two cases are omitted, the range of cholinesterase values for the group becomes 0.59 to 1.12 for the erythrocytes and 0.47 to 1.60 for the plasma. The average values for the red cells and the plasma are increased to 0.71 and 0.89, respectively. All these values are within the normal limits. Persons living far from orchards who had no known contact and no obvious potential contact with organic phosphorus insecticides showed a negligible depletion of cholinesterase as compared with average normal values.

It is worthy of note that the lowest cholinesterase values observed were obtained from a part-time applicator (Case 15-51-B-215) whose near fatal illness is described in some detail below. Instances of this type, as well as other kinds of accidents encountered in the study or reported by other authors, serve as a warning that no interpretation of the toxicity of the organic phosphorus insecticides can be accurate without taking the elements of carelessness and human error into account. The figures clearly show that the average cholinesterase values of the persons receiving the greatest average day-to-day exposure were lower than the average values of other groups. In spite of this, the only fatal and near-fatal cases which were found were associated not with long-standing exposure but with gross carelessness.

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[6] Johnston, J. M.: Parathion Poisoning in Children, J. Pediat. 42:286, 1953.

Most of the workers studied used some protective clothing, especially when their exposure was greatest. The use of complete protective clothing was very uncommon, though, and this fact must be taken into account in evaluating what is meant by gross carelessness.

Another method for estimating the effect of exposure is to measure the maximum depletion of cholinesterase shown during the year. Information of this kind is shown in Table 2 for 56 persons studied during 1951 who provided a blood sample during the preexposure period and at least one other sample during the exposure period. The lowest red blood cell and corresponding plasma cholinesterase value shown by each person during his exposure period was subtracted from the appropriate value for the preexposure period. In certain instances, the lowest value during the exposure period was higher than that for the preexposure period. This is indicated in the Table by a plus sign in front of the appropriate figures. In every instance these increases were quite small, and they undoubtedly represent a biological variation rather than any effect of chemicals. The changes recorded in the Table were not dramatic even for commercial applicators and part-time applicators, but it must be recalled that this Table is limited to those persons whom it was possible to follow over a period of time. The Table automatically excludes those persons who were seriously poisoned (for example, Case 15-51-B-215) and who came to a physician's attention only after they had become ill and as a direct result of the illness. However, Table 2 does serve to show that, on the average, significant changes in cholinesterase values were restricted to those occupational groups with known exposure to the organic phosphorus insecticides. It does appear that some persons living near orchards experienced a statistically significant, though not dangerous, depletion of their cholinesterase values during the spray season. However, as is evident from Table 1 and Chart 1, only a few values were depressed beyond the range which is thought to represent normal biological variation.

TABLE 2.—*Maximum Change in Cholinesterase Values of Different Occupational and Check Groups* *

Occupational Group	No. of Persons	Maximum Change in Cholinesterase Values (Δ pH/Hr.)					
		Red Blood Cells			Plasma		
		Range	Mean	Std. Error	Range	Mean	Std. Error
CA	7	−0.06 to −0.27	−0.19	0.06	+0.01 to −0.33	−0.12	0.04
PTA	15	+0.01 to −0.17	−0.07	0.01	+0.13 to −0.48	−0.08	0.08
WO	8	+0.08 to −0.32	−0.08	0.04	+0.12 to −0.31	−0.05	0.04
FWM	5	+0.04 to −0.08	−0.04	0.05	+0.07 to −0.26	−0.06	0.06
RNO	21†	+0.15 to −0.33	−0.03	0.04	+0.33 to −0.30	−0.01	0.02

* These values were determined by subtracting the lowest value for each person during the exposure period from his preexposure value. When a decrease occurred, the change was counted negative. When the cholinesterase value increased, even though there had been a potential exposure, the change was counted positive.

† Only 20 persons could be evaluated for erythrocyte values.

Inspection of the portions of Table 1 dealing with the recovery and postrecovery periods indicates that any failure of recovery which may be indicated was small.

The average values which are low were derived in each instance from a small number of samples and probably represent no significant deviation from normal. Any failure of complete recovery undoubtedy is explained by the known slowness in the regeneration of cholinesterase, especially in the red blood cells. That recovery is eventually complete is indicated by Chart 1. In this Chart the observed red cell cholinesterase values are plotted against time for mixing-plant personnel and for residents living near orchards. In plotting the points, no account has been taken of the personal exposure history of the persons involved. The observed values were merely plotted against the date on which each sample was taken. This graph serves to show that the depletion of red cell cholinesterase among mixing-plant personnel was a gradual process and that recovery was likewise gradual. The depletion at the middle of August was striking and essentially uniform. The highest value for red cell cholinesterase observed in five workers sampled on Aug. 14 was 0.34; the lowest value was 0.11. In contrast to the trend among mixing-plant personnel, no distinct trend toward depletion could be observed in the cholinesterase values of persons who resided near orchards but who had no known occupational contact with organic phosphorus insecticides. This is emphasized by the fact that the two very low values observed for residents living near orchards during October were taken from one person who had been accidentally and rather severely poisoned.

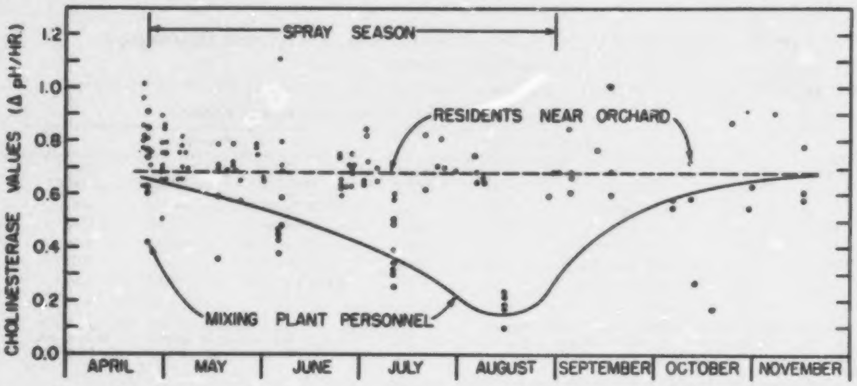

Chart 1.—Graph showing red cell cholinesterase values for mixing-plant personnel and residents living near orchards. The curves represent approximate trends. The extent of the organic phosphorus insecticide spray season is indicated. The two very low values observed in residents living near orchards during October were taken from one person who had been accidentally and rather severely poisoned.

Symptomatology.—In reviewing the data, the 59 symptoms which were recorded one or more times were tabulated according to occupational groups and exposure periods. Some of these symptoms were associated with hypertrophy of the prostate, presbyopia, stomach ulcer, and a number of other established diagnoses in a few of the subjects. The original tabulation served to confirm the lack of any correlation between this class of symptoms and exposure to organic phosphorus insecticides, and they were omitted from the itemized portion of Table 3 as presented. They are included, however, among the "total number of illnesses" and are thus reflected in the "percentage of persons with illness," as recorded in that Table. Under the

TABLE 3.—*Occurrence of Any Illness and of Certain Selected Symptoms Among Different Occupational and Check Groups as Related to Exposure to Organic Phosphorus Insecticides**

Occupational Group	No. of Persons	No. of Illnesses	Percentage of Observed Illnesses Exhibiting Selected Symptoms: Headache	Nausea	Weakness or Fatigue	Pain or Tightness in Chest	Abdominal Pain	Vertigo, Fainting, or Incoordination	Vomiting	Nervousness, Drowsiness, or Insomnia	Sweating	Cough or Expectoration	Disturbance of Vision	Loss of Appetite	Shortness of Breath	Nasal Discharge Including Postnasal Drip	Myosis	Wheeze	Percentage of Persons with Illness	Percentage of Illnesses Exhibiting at Least One Selected Symptom	Percentage of Illnesses Exhibiting Myosis or at Least 3 Other Selected Symptoms
Preexposure																					
MPP	8	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—	—
CA	23	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	9	0	0
PTA	28	5	..	..	..	..	..	..	..	..	..	..	20	..	20	..	..	..	18	40	0
WO	17	1	..	..	..	..	..	..	..	..	..	..	..	..	..	100	..	..	6	100	0
FWM	11	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	18	0	0
RNO	30	3	..	..	33	..	..	..	..	..	..	..	..	..	..	33	..	..	10	33	0
RFO	1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—	—
ROA	15	12	17	..	..	..	17	..	..	17	..	..	..	..	..	..	..	..	33	41	0
Exposure																					
MPP	10	5	80	60	20	20	20	..	20	..	20	20	..	..	20	20	..	20	50	100	80
CA	43	40	60	25	30	27	30	10	10	7	7	18	15	10	15	7	10	7	70†	97	60
PTA	50	37	57	27	24	19	19	27	11	8	14	11	5	14	11	11	3	8	50‡	84	30
WO	15	7	43	..	14	14	..	..	..	..	..	29	..	..	14	14	..	..	47	86	14
FWM	14	5	20	..	20	..	20	20	20	..	..	..	..	..	..	20	20	..	36	60	40
RNO§	31	9	44	22	22	22	..	22	22	11	..	33	..	..	11	..	..	..	29	89	44
RFO	1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—	—
Recovery																					
MPP	5	1	..	100	..	..	100	..	..	..	..	..	..	..	..	..	..	..	20	100	0
CA	18	2	..	..	..	..	50	..	..	..	..	..	..	..	..	..	..	..	11	50	0
PTA	14	4	25	25	25	..	25	25	..	25	..	..	..	..	25	..	..	..	29	75	25
WO	8	2	..	..	..	..	..	..	..	..	..	..	..	..	..	50	..	..	25	50	0
FWM	3	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—	—
Postrecovery																					
MPP	4	1	..	100	..	..	100	..	..	..	..	..	..	..	..	..	..	..	25	100	0
CA	17	1	..	..	..	..	100	..	..	..	..	..	..	..	..	..	..	..	6	100	0
PTA	7	1	..	..	..	..	..	..	..	..	..	..	..	..	100	..	..	..	14	0	0
WO	1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—	—
FWM	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	33	0	0
RNO	3	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—	—
RFO	2	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	100	0	0

* All values in the Table are zero unless otherwise indicated.
† Of the 43 persons involved, 30 (70%) suffered a total of 40 separate illnesses.
‡ Of the 50 persons involved, 25 (50%) suffered 37 illnesses.
§ A case of fatal poisoning mentioned in the text has been omitted from this tabulation.

circumstances, however, the tabulation based on total illness only serves to show that during each exposure period the various occupational groups were questioned in sufficient detail to reveal complaints in a high proportion of the subjects. The distribution of illness, without further classification, gives little or no hint of the effect of exposure.

For the purpose of further analysis, 23 of the symptoms from the complete list were combined under 16 headings. The selection was based on the following: (1) inclusion of symptoms with known or suspected association with intoxication; (2) exclusion of signs, such as convulsion or coma, encountered only in serious poisoning, and (3) exclusion of certain signs or symptoms, such as dermatitis, which appear not to represent a systemic effect even when they do seem to be the result of exposure to organic phosphorus insecticides. The selected symptoms are listed in the column heads of Table 3. When the percentages of observed illnesses involving at least one of these symptoms were tabulated, they showed a random distribution in regard to the exposure periods and the occupational groups. However, when those illnesses involving myosis or at least three of the other selected symptoms were tabulated, the picture was entirely different. No such illness was encountered during the preexposure or the postrecovery period, and the single instance during the recovery period is adequately explained by the fact that the subject was no longer exposed but was still suffering from poisoning. In the case of the exposure period, on the contrary, at least a part of all the illnesses of each occupational group during this period was illness of this particular character. There is, then, strong evidence that illness characterized by myosis or by any three of the selected symptoms was increased in the definitely exposed groups during the period of their exposure. A few instances of obvious intoxication were included. Review of the individual cases revealed that these instances of obvious intoxication and most of the characteristic mild illnesses also bore a definite and convincing relationship to exposure. This was true even though, in general, it was impossible to distinguish the individual grouping of symptoms which these 38 illnesses demonstrated from groupings which might occur in the total absence of exposure to organic phosphorus insecticides. Therefore, the absence of a small proportion of "characteristic" illnesses during periods when there was no exposure must be regarded as accidental. On the contrary, it is very likely that some illnesses encountered in this investigation were the direct result of exposure, but they failed to meet the arbitrary criteria mentioned above which were established merely for the purpose of study. For example, of the 103 illnesses encountered during the exposure period, 65 were characterized by headache, 29 by nausea, and 26 by weakness or fatigue. Only 3 instances of headache, 3 of nausea, and 2 of weakness and fatigue were encountered among 40 illnesses found during periods of nonexposure. Even of this small number, one headache, two instances of nausea, and one instance of weakness or fatigue occurred during the recovery period in persons no longer exposed to insecticides but still suffering from recognized poisoning.

Viewed another way, it was possible to account completely for 31 (78%) of the 40 illnesses encountered during periods of nonexposure without the use of the diagnosis of intoxication; with the use of the same criteria, it was possible to account for only 10 (10%) of the 103 illnesses encountered during the exposure period. For example, abdominal pain associated with diagnosed peptic ulcer was considered "accounted for," but abdominal pain not associated with this or some other adequate cause was considered "unaccounted for."

Although, as already stated, the relationship between exposure and disease (both mild and severe) is considered significant, the method by which the data were gathered and particularly the small total number of illnesses which were available for analysis make it unwise to ascribe much significance to the exact percentages of illness which were recorded. Furthermore, although the statistical evidence for an increased incidence of characteristic illness was confirmed by clinical judgment of most of the individual cases of mixing-plant personnel, commercial applicators, and others with significant exposure, this is not true of the residents living near orchards who did not experience gross, accidental exposure. The number of illnesses thought to resemble mild poisoning in residents near orchards was small, and these few illnesses did not present a clear and convincing relationship to exposure. The statistical evidence for an increase in mild disease in residents near orchards resulting from their exposure to organic phosphorus insecticides must therefore be regarded as inconclusive. Of course, it is obvious that the risk of accidental poisoning is greater in homes located in orchards than in other homes, because there is a greater chance of gross contamination. Instances in which children have found containers of concentrate left near the house illustrate this point.

The morbidity in different occupational groups induced by exposure to the organic phosphorus insecticides may be determined later by an epidemiological survey. Such a survey would involve the taking of blood samples in only special instances. The drawing of blood under field conditions for a large number of samples occupies a surprising amount of the physician's time; more important, it serves to limit the number of volunteers available for long-time study.

Most of the workers who suffered mild illness as the result of exposure continued work. Examples occurred of definite symptomatic improvement in the face of continued exposure. In some instances but not in all, this improvement was conditioned by the adoption of protective clothing which consequently led to reduced exposure. A few workers sought other employment as the direct result of illness. This suggested but in no way proved that some variation in susceptibility existed and that the more susceptible persons tended to select work which involved no exposure to insecticides.

In the 38 illnesses during the exposure period which involved myosis or at least three of the other selected symptoms, the different symptoms occurred the following number of times: headache, 29; nausea, 21; weakness or fatigue, 19; pain or tightness in the chest, 17; abdominal pain, 13; vertigo, fainting, or incoordination, 13; vomiting, 11; nervousness, drowsiness, or insomnia, 9; sweating, 9; cough or expectoration, 9; disturbance of vision, 9; loss of appetite, 8; shortness of breath, 8; nasal discharge including postnasal drip, 6; myosis, 5, and wheeze, 5. In four of the five instances of myosis, at least three other selected symptoms were also present. The same 38 illnesses mentioned above also involved certain other symptoms possibly related to poisoning, as follows: fever, 6; weight loss, 5; muscle pain, 4; twitching, paresthesia, change in the sensation of taste, and nosebleed, 3 each; loss of ability to concentrate, heart consciousness, food intolerance, gas, and chills, 2 each, and photophobia and diarrhea, 1 each. A few of the same patients also wore glasses, had masses or glands, or suffered from hemorrhoids—all situations obviously unrelated to possible poisoning. Of the six instances of fever, three appeared to be accounted for by intercurrent infection, but the cause was not determined in the others. Gas and food intolerance, although listed above, appeared to be related to preexisting chronic disease.

Only five instances of myosis were observed, although it is possible that myosis was present at the time of illness in some other subjects. In one case myosis was the only sign or symptom present, and difficulty in vision was not noted. Of four persons with myosis and other symptoms, one did not complain of disturbance of vision; the other three complained of some visual disturbance, and one, a pilot, complained specifically of photophobia, difficulty in judging distances, and diplopia, the ceiling of a room appearing to this man to be very much higher than it actually was. In one instance, a pilot complained of visual disturbance resulting from exposure but at a time when he showed no myosis. He stated that even in bright sunlight his environment appeared "as if it were dusk." He had particular difficulty in the evening. He attributed the effect to tetraethylpyrophosphate and not to parathion. It appears from this and from later observations that visual disturbance from organic phosphorus insecticides may have a complex physiological basis and cannot be judged or predicted dependably from objective examination of the pupil. Workers commonly held the opinion that tetraethylpyrophosphate was much more likely to cause visual disturbance than parathion. Although the workers were exposed to both compounds, and there was no opportuity for a controlled experiment, there was no reason to doubt the judgment of the men themselves.

Four persons under study complained of dermatitis. Of the four, two had known exposure. One of them suffered an erythema of the hands and face after extensive exposure. The relationship appeared clear-cut. Another patient had a chronic, intermittent, eczematous dermatitis which he thought was due to exposure. He admitted during hospitalization that several of his other difficulties began when his private physician started giving him inoculations of liver extract, a material which is frequently sensitizing. Pathological findings in a skin biopsy were reported consistent with colloid degeneration. Although it was not possible to determine absolutely the cause of the dermatitis, there appeared to be little reason to incriminate organic phosphorus insecticides.

Two subjects complained of dermatitis without any known exposure. One had a recognized case of fungus infection. The case of the other could not be diagnosed with certainty, but insecticides appeared a very unlikely etiology, because there was no known exposure and the eczema continued for weeks after insecticide applications were discontinued in the autumn throughout the whole area.

Relationship of Symptomatology and Cholinesterase Level.—In Table 4 are presented the cholinesterase values of blood samples taken near the time of illness from persons exhibiting myosis and/or any three of the selected symptoms of organic phosphorus insecticide poisoning. It may be seen that the average values for the different occupational groups were, with two exceptions, lower than the corresponding values in Table 1. On the average, the subjects who suffered probable poisoning did so when their cholinesterase values had already undergone the greater part of the depression which they ultimately showed. Also, as one would expect, the ranges indicate that characteristic illness was encountered in the face of very low cholinesterase values.

What is of greater interest, because it has not been demonstrated adequately before, is that, on the average, the characteristic illness occurred in the presence of cholinesterase values which fall within the range of normal. Taken individually, many of the values could not be surely distinguished from normal even though, when presented as groups, the averages were subnormal. Where a direct comparison was possible, persons with even mild symptoms were found to show a decrease in cholinesterase values at the time of illness, as contrasted with their individual pre-exposure values. However, it is clearly demonstrated that the mere fact that a cholinesterase value is within the normal range cannot be used to exclude the diagnosis of organic phosphorus insecticide poisoning, although it does exclude serious poisoning from that source.

On the other hand, some workers showed late-season erythrocyte and plasma cholinesterase values as low as 0.11 without complaining of any symptom throughout the season. In these instances, the low values were reached slowly and only at the end of the exposure season.

TABLE 4.—*Cholinesterase Values Near Time of Illness of Persons Exhibiting Myosis or at Least Three Other Selected Symptoms* *

Occupational Group	No. of Persons	No. of Persons with Characteristic Illness	Cholinesterase Values (Δ pH/Hr.)			
			Red Blood Cells		Plasma	
			Range	Mean	Range	Mean
MPP	10	4	0.31 to 0.76	0.50	0.30 to 0.68	0.37
CA	42	16	0.19 to 0.76	0.39	0.24 to 1.08	0.57
PTA	59	11	0.09 to 0.92	0.59	0.08 to 0.97	0.47
WO	15	1	0.51	0.51	0.56	0.56
FWM	14	2	0.61 to 0.71	0.66	0.40 to 0.71	0.56
RNO	21	4	0.60 to 0.70	0.64	0.85 to 1.19	1.04
RFO	1	0	....	....	....	....
ROA	0	0	....	....	....	....

* The values are all related to the period of exposure; a single person still showed characteristic illness during the first few days of his recovery period.

REPORT OF CASES

The cases of children involved in the present study have been reported separately.[6] The following cases are also of interest. Since some of these patients represented medical emergencies, the initial clinical observations which were generously supplied to us were understandably brief.

CASE 15-51-B-215.—R. S. was a 24-year-old white man, a graduate student in agriculture. Like many students, he took a summer job relating to his intended profession but inevitably involving some menial labor. During his first week in Wenatchee, Wash., he was assigned to various painting jobs. On July 23, 1952, he was placed in a crew to help spray an orchard. He handled a nozzle from a stationary power sprayer. The insecticide formula consisted of 6 lb. of a 25% parathion wettable powder and 9 lb. of a 50% D.D.T. wettable powder, added to 600 gal. of water and sprayed at the rate of 1,700 gal. per acre or approximately 20 gal. per tree. A sprayer can average about 75 trees a day.

R. S. worked Monday, July 23, and Tuesday morning. The wind prevented spraying on Tuesday afternoon and all day on Wednesday. Thursday morning was very hot and there was no wind, so spraying was resumed. By noon Thursday, July 26, the student was unbearably hot, so he discarded his raincoat and respirator and finished spraying during the rest of the afternoon with only his pants and boots as protection. He became soaked with the formula.

He went home and ate a good dinner. About 6:30 p. m., immediately after dinner, he complained of "light-headedness" and a very slight headache accompanied by dizziness. He went to bed and immediately started vomiting. He "passed out" several times after falling on the floor, and he was unable to gather enough strength to pull himself up on the bed. Perspiration was profuse, and the patient suffered muscle and abdominal cramps, tightness in the chest, and blurred vision. His wife called a nurse who lived next door and who diagnosed his case as "spray poisoning." The nurse gave him atropine sulfate, 1/100 grain (0.6 mg.) by mouth, and called a local physician who ordered him to the hospital. He was admitted at 9:00 p. m. By this time the patient was in a shock-like condition and disoriented.

His temperature was 99.4 F.; pulse rate, 56 and irregular, and the blood pressure, 138/88. His condition was described as "dyspneic" even though his respirations were only 12 per minute at the time. (At 9:45 p. m. the respiratory rate was 28.) He was acutely ill and semicomatose. The skin was pale and drenched in perspiration. The pupils were small. The tongue was dry. The lungs showed a few abnormal sounds but no dullness. The heart was slow and irregular, and the sounds were of variable intensity. No murmurs were detected. The abdomen showed generalized tenderness but no masses or distention. The reflexes were intact, but the patient showed diminished muscular power, and such movements as were attempted were poorly coordinated.

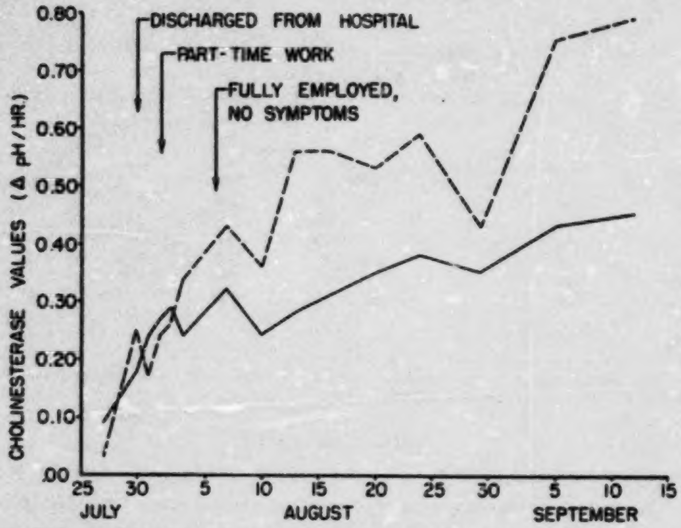

Chart 2.—Plasma (broken line) and red cell (solid line) cholinesterase values for Case 15-51-B-215, showing recovery after severe poisoning.

On admission to the hospital the patient was treated with atropine sulfate, 1/50 grain (1.2 mg.), intramuscularly, and he received 3 doses of 1/100 grain between 9:50 p. m. and 11:30 p. m. No further atropine sulfate was given until the next afternoon, when a dosage of 1/100 grain every six hours was begun. This dosage was continued until discharge. Oxygen was given shortly after admission and its use discontinued the next morning. Caffeine as a respiratory stimulant and meperidine (Demerol) hydrochloride for the relief of cramps were given shortly after admission and were not repeated. Phenobarbital sodium was given at bedtime on July 27 and thereafter. A 500 cc. bottle of 10% dextrose in isotonic saline solution was administered shortly after admission, and administration of another 1000 cc. started at noon the next day.

On the morning of July 27, the vomiting continued; the patient was disoriented and completely exhausted; his extraocular movements were pathological. He complained of cramps in his legs and abdomen and excessive sweating. He said he felt "as if my stomach was on the move all the time." These symptoms all gradually improved that day, and by evening he retained his first meal.

The temperature rose gradually to 100 F. on the evening of the second day (July 27); it was essentially normal during the remainder of the hospital course. The pulse remained normal or slightly slow and became regular by July 28. The respiratory rate returned to normal by the morning of the second day and remained normal. No increase in blood pressure was observed; the lowest values seen were 98/60 and 96/62 on July 27 and 29, respectively. There was a leucocytosis of 13,450, with 87% polymorphonuclear leucocytes on the evening of admission. On the following day the urine showed 4+ reactions for sugar and a specific gravity of 1.026, probably as a result of the infusions. Other general laboratory studies were normal.

The patient was discharged from the hospital the morning of July 30. He still complained of weakness, headache, and some nausea. One week after hospitalization the only residual symptom was headache, which was still present on Aug. 3. He returned to work for the first time for a part of Aug. 2, and he worked a part of Aug. 3 also. By Aug. 7, 12 days after the initial illness, he worked full time and had no symptoms. He remained asymptomatic.

His cholinesterase values are reported in Chart 2.

Case 15-51-B-26.—C. L. S. was an 18-year-old white laborer in an insecticide mixing plant. A blood sample was taken from him at 8:30 the first morning he reported for work. He wore a double canister respirator while he worked at putting finished 25% parathion wettable powder into sacks. At 4 o'clock in the afternoon he became ill and complained of headache, nausea, vomiting, and profuse sweating. On examination, he appeared quite ill, and there were coarse rales throughout both lung fields. He was pale and sweaty. The pulse rate was 100, and the blood pressure was 100/60. There was no myosis. The patient was given 1/100 grain of atropine sulfate and sent home. At 7:00 p. m. a second blood sample was taken. The samples were analyzed by a titrimetric method. The erythrocyte and plasma values showed a reduction of 18 and 16%, respectively. The patient recovered sufficiently to leave town the next day to seek a different kind of employment.

Table 5.—*Cholinesterase Values in Case 15-51-B-58, the Patient Showing No Symptoms When Values Were Lowest*

Date	Cholinesterase Levels	
	Red Blood Cells	Plasma
April 25, 1951	0.86	0.41
June 6, 1951	0.46	0.30
July 11, 1951	0.30	0.14
Aug. 14, 1951	0.19	0.14
Oct. 8, 1951	0.57	0.52
Dec. 17, 1951	0.79	0.54
Jan. 14, 1952	0.82	0.55
Feb. 6, 1952	0.77	0.52

Case 15-51-B-58.—B. W. L. was a 45-year-old white laborer in a commercial mixing plant. He was not exposed to organic phosphorus insecticides during 1950. His first blood sample for cholinesterase determination in 1951 was taken after he had already worked for seven days. From the middle of April until the last of August he worked eight hours a day, six days a week. He wore gloves and overalls regularly but wore a mask only while sacking dust. His clothes became very dusty during the day. He changed clothing and bathed each evening. This man had no spontaneous complaint, but questioning early in the season revealed that he could not eat spicy foods and that he suffered occasional nausea and abdominal pain associated with a recognized peptic ulcer for which he was under treatment. During early June he stated that he had occasional headaches but doubted that they were caused by parathion or tetraethylpyrophosphate. During July and August he denied any symptoms. The case is interesting especially because of the low cholinesterase values which were reached in the absence of symptoms. The enzyme values are shown in Table 5. Plasma enzyme values lower than erythrocyte enzyme values are seen in some subjects who have had no exposure and who have no recognized disease. The presence of this relationship in the blood chemistry of B. W. L. five months after the last exposure was therefore probably not evidence of a failure to recover but merely an illustration of normal variation.

COMMENT

It must be emphasized that all available evidence indicates that even mild organic phosphorus insecticide poisoning is usually, if not always, accompanied by a decrease in cholinesterase values. However, in mild illness, it frequently happens that a cholinesterase value which is significantly low for an individual person is still well within the range of values for the completely unexposed, normal population. It is because of this circumstance that a single cholinesterase determination that falls within the range of normal cannot be used to completely exclude a diagnosis of mild intoxication in a patient who presents himself for the first time. A careful medical history and a general medical examination will do much to distinguish poisoning of any degree whatsoever from other illness. Proper supervision of workers demands this kind of study, as well as the determination of enzyme levels.

Some workers develop extremely low cholinesterase values by the end of a long period of exposure without experiencing any symptoms. This fact and the circumstances mentioned in the preceding paragraph make it very difficult to establish an arbitrary critical value which would represent a "safe" level for exposed workers. Apparently Grob and his colleagues [7] were the first to employ "periodic determinations of plasma and red blood cell cholinesterase activity in order to detect those employees who have absorbed [parathion]." The authors stated that workers with reduced cholinesterase activity had been removed from all exposure to parathion until their enzyme values returned to normal. The authors did not discuss the limits of normalcy.

Marchand [8] has stated that person-to-person variations may reach plus or minus 20% for red cell values and plus 100 or minus 20% for plasma values. This variability is measured against an average for an unexposed population. Without discussing the exact degree of variability to be found in an unexposed population, it is clear from the present report that a substantial proportion of persons who become mildly ill as the result of exposure will not at the time show more than a 20% reduction in their cholinesterase values as compared with an acceptable, average, normal value derived from an unexposed population. Even the average red cell cholinesterase values shown in Table 4 for persons who were ill as the result of exposure show only about a 20% reduction as compared with 0.77, the average preexposure value found in Wenatchee. This is true, even though Table 4 contains values for persons who were severely poisoned as well as for those whose cases were mild.

It has been suggested by Barnes and Davies [9] that other symptoms besides myosis may represent local effects resulting from contact with air-borne particles. It appears certain from experience with man, as well as from tests on animals, that especially the more soluble organic phosphorus insecticides may produce contraction of the pupil when applied directly to the eye. Although the circulation of the iris is peculiar, it is possible that other organs and tissues may be capable of showing local effects also. If this could be demonstrated objectively under conditions similar to those met by workers, it might form a dependable explanation for much of the mild illness encountered in this study. However, it is difficult to believe that sickness from systemic poisoning associated with general circulation of the toxin in the body does not vary from fatal attacks to the mildest symptoms. As mentioned earlier, no clear-cut dividing line between severe and mild cases was detected in the present study. In this connection, one may speculate that mild symptoms which represent systemic effects are the result of a rapid but relatively small reduction in cholinesterase values. The severe or even fatal illnesses seen in patients in Wenatchee involved brief exposure, and the reduction in cholinesterase values was rapid as well as great. However, experiments demonstrate that fatal illness may occur suddenly in an animal which has been maintained at a low cholinesterase value for months. Death in such an animal frequently, if not always, is accompanied by a slight additional fall in the enzyme levels.

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[7] Grob, D.; Garlick, W. L.; Merrill, G. G., and Freimuth, H. C.: Death Due to Parathion. an Anticholinesterase Insecticide, Ann. Int. Med. **31**:899, 1949.

[8] Marchand, J. F.: Microtests for Cholinesterase: Interpretation After Nerve Gas or Agricultural Insecticide Exposure, J. A. M. A. **149**:738, 1952.

[9] Barnes, J. M., and Davies, D. R.: Blood Cholinesterase Levels in Workers Exposed to Organo-Phosphorus Insecticides, Brit. M. J. **2**:816, 1951.

Two valuable contributions of the cholinesterase determination should be emphasized. The first contribution is in clinical medicine when the values are sufficiently depressed that they are of use in diagnosis; this is always true in severe poisoning. The second contribution has been mentioned by Marchand,[8] and he and his colleagues have earlier brought the information by a large number of informal releases to the factory and agricultural supervisors who can use it. This information is namely: "When groups are screened, the extent that individual values or the averages for the group as a whole fall below the preexposure controls or normal averages serves as an objective index of the need for correction of handling methods or protective routines."

SUMMARY AND CONCLUSIONS

1. In 1951, 258 persons with different degrees of chiefly agricultural exposure to organic phosphorus insecticides were studied in regard to blood cholinesterase values and symptomatology.

2. The average cholinesterase values for groups known to have definite and consistent exposure showed significant reduction during the period of exposure. Average erythrocyte enzyme values for mixing-plant personnel, commercial applicators, and part-time applicators were 0.46, 0.65, and 0.69, respectively, as compared with 0.72, the lowest average value found for any group during the preexposure period.

3. The average cholinesterase values for groups with little or no exposure to insecticides remained normal or showed changes so small that their significance is in doubt.

4. Although extensive day-to-day exposure was regularly associated with reduced blood cholinesterase, the only fatal and near-fatal cases which were found were associated not with long-standing exposure but with brief, massive exposure and gross carelessness.

5. Illness characterized by myosis or by any three of a group of selected symptoms was considered suggestive of poisoning but not absolutely diagnostic of it in isolated cases. Such illness was increased in the definitely exposed groups during the period of their exposure.

6. Illness characterized by myosis or by any three of a group of selected symptoms is essentially diagnostic of poisoning when accompanied by a significant reduction in the cholinesterase values.

7. Most workers who suffered mild illness as a result of exposure continued to work. Examples occurred of definite symptomatic improvement in the face of continued exposure. A few workers sought other employment as a direct result of illness.

8. Some evidence was obtained that visual disturbance caused by organic phosphorus insecticides may have a complex physiological basis and may not be the direct and simple function of myosis.

9. Serious poisoning was not encountered in the absence of very severe depletion of cholinesterase values or in the absence of excessive exposure.

10. Mild characteristic illness occurred in the presence of cholinesterase values which fall within the range of normal for the population as a whole. It is possible and even probable that illness as the direct result of exposure did not occur in any person without a decrease in his cholinesterase values as compared with his own personal normal values. Even so, a single normal determination cannot be used to exclude the diagnosis of organic phosphorus insecticide poisoning, although it does exclude serious poisoning from that source.

11. Some workers showed enzyme values as low as 0.11 without complaining of any symptom at any time during the season.

12. The relationship of cholinesterase to illness and fatality is discussed briefly, and illustrative case histories are given.

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Polychlorinated Biphenyls: Evidence of Transplacental Passage in the Sherman Rat

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Summary—The polychlorinated biphenyl (PCB), Aroclor 1254, was given orally in peanut oil to pregnant Sherman rats once daily from day 7 to day 15 of organogenesis. Dose levels were 0, 10 and 50 mg/kg/day. No statistical difference was found between control and dosed groups with respect to the total weight of litters, the percentage of pups born dead or the survival rate to weaning. Residue levels of PCB-derived material were measured in the foetuses, in the milk ingested by sucklings and in tissues of weanlings by electron-capture gas–liquid chromatography. Liver enlargement was observed in weanlings from dosed rats, but not in those from controls. There was a significant increase in the relative liver weights of weanlings from dosed rats compared with those from controls and the livers of most of the exposed weanlings contained enlarged hepatocytes, accompanied, in some cases, by cytoplasmic vacuolization and bile-duct proliferation, particularly in the group given the higher dosage.

Introduction

A previous study of oral dosing and dietary ingestion of Aroclor 1254* in Sherman rats demonstrated a wide distribution of material derived from chlorinated biphenyls (PCBs) in all tissues and excreta (Curley, Burse, Grim, Jennings & Linder, 1971). Other researchers have shown that some PCBs can induce microsomal-enzyme activity (Risebrough, Rieche, Peakall, Herman & Kirven, 1968) and cause porphyria (Vos & Koeman, 1970). The recent and persistent reports of PCBs in birds (Bagley, Reichel & Cromartie, 1970), fish (Risebrough *et al.* 1968) and man (Biros, Walker & Medbery, 1970) suggest that these compounds are as ubiquitous in the environment as *p,p'*-DDE.

Concentrations of *p,p'*-DDE, *p,p'*-DDT and other chlorinated hydrocarbons have been determined in human placentas and cord blood (O'Leary, Davies & Edmundson, 1970; Rappolt & Hale, 1968). The distribution of [^{14}C]DDT and dieldrin in pregnant mice (Backstrom, Hansson & Ullberg, 1965) and observable effects on the offspring resulting from the feeding of mirex to pregnant rats (Gaines & Kimbrough, 1970) all substantiate that transplacental passage of pesticides can occur.

The recent interest in PCBs and the transplacental movement exhibited by lipoid-soluble chlorinated compounds prompted this study. This paper reports the results of oral administration of Aroclor 1254 to pregnant rats and its subsequent detection in foetuses, weanlings and milk.

*Aroclor is a registered trade-name of Monsanto Co., St. Louis, USA.

Experimental

Three groups of female 90-day-old Sherman rats (mean body weight 254 g) were pair-mated with male rats of the same age. A vaginal smear from each female was examined microscopically each day until insemination was verified. Then male rats were removed. All rats were maintained on a diet of plain chow and water.

Considering the day of insemination as day 0, rats were given a single oral dose of Aroclor 1254 in peanut oil once a day on days 7–15 (inclusive) of organogenesis. The levels of dosage were 0, 10 and 50 mg/kg for groups 1, 2 and 3, respectively.

On day 20 of pregnancy, foetuses were taken by Caesarean section from three rats in each treated group and from two control rats. Foetuses from each dam were divided into two groups and analysed for PCB content as duplicate samples.

Ten dams in each group were allowed to deliver spontaneously. The litters were weighed, only the live rats being weighed in each group. When the pups were 5 days old, six mothers from each dosed group and three from the control group were removed from their offspring for several hours and then returned to their respective litters for 1 hr of nursing. Stomach content (milk) of pups was removed and pooled for each respective litter.

The litters of three mothers in each group were allowed to survive until weaning (21 days old). One male and one female weanling from each mother were weighed and sacrificed and the liver, brain and kidney were taken for analysis. The livers were weighed and sections of the liver from the other weanlings were examined microscopically.

Combined foetuses weighing 20·0 g were extracted with 200 ml hexane in an Omni-mixer for 5 min and filtered through a glass-wool plug into a 250 ml graduated cylinder. An aliquot was taken, the equivalent of 1·0 g. Milk (1·0 g) was homogenzied in 10 ml hexane. The extraction of other tissues including liver, kidney and brain, and the details of sample preparation, clean-up and analysis by electron-capture gas–liquid chromatography have been described previously (Curley *et al.* 1971).

Results

For animals subjected to Caesarean section on day 20, the control group averaged nine foetuses/dam while the dosed groups averaged ten foetuses/dam. All foetuses were normal upon gross examination. Of the dams allowed to deliver spontaneously, group 1 averaged 11·8 pups and 67·6 g/litter with none born dead, group 2 averaged 12·6 pups and 72·7 g/litter with one born dead and group 3 averaged 12·9 pups and 67·3 g/litter with four born dead.

Table 1 shows the concentrations of PCB-derived components found in foetuses taken by Caesarean section on day 20 of pregnancy. Measurable quantities of PCB-derived components were found in all samples except controls.

Although a fivefold difference in dosage level existed between group 2 given 10 mg/kg daily for 9 days and group 3 given 50 mg/kg daily for 9 days during organogenesis, the difference between the mean concentration of PCB in foetuses was only twofold. Residue levels in group 2 differed significantly ($P < 0{\cdot}001$) from those in group 3.

Table 2 shows the concentrations of PCB-derived components found in milk samples taken from suckling rats 11 days after the last dose was administered to the mother. PCB-derived components were found in all the milk samples analysed except those from the controls. There was a statistically significant difference ($P < 0{\cdot}001$) between groups 2 and 3. The mean concentrations of PCB in milk for the two treated groups showed a threefold difference for a fivefold difference in dosage.

Table 1. *Concentrations of PCB-derived components found in foetuses from rats given an oral dose of Aroclor 1254 on days 7–15 of pregnancy*

PCB dose (mg/kg/day)	No. of adult females	Mean body weight (g)	Level of PCB-derived components in foetuses (μg/g)*
0	2	253	< 0·12
10	3	258	0·63 ± 0·06 (0·40–0·78)
50	3	252	1·38 ± 0·06 (1·20–1·55)

*The values for the two dosed groups are means ± SEM for six determinations, with the range given in parenthesis.

Table 2. *Concentrations of PCB-derived components found in litter-pooled milk taken from suckling rats born to dams given an oral dose of Aroclor 1254 on days 7–15 of pregnancy*

PCB dose (mg/kg/day)	No. of adult females	Mean no. of pups/pooled sample	Level of PCB-derived components in the milk (μg/g)†
0	3	12	< 0·75
10	6	12·5	20·60 ± 1·59 (16·48–24·90)
50	6	12·3	66·34 ± 8·36* (45·80–100·29)

†The values for the two dosed groups are means ± SEM for six pooled samples, with the range given in parenthesis. The value marked with an asterisk differs significantly (Student's *t* test) from that for the other dosed group: *$P < 0{\cdot}001$.

The average survival rate to weaning was 93·5, 96·0 and 90·0% for groups 1, 2 and 3, respectively. Table 3 summarizes the mean concentrations of PCB-derived components found in selected tissue in 21-day-old weanlings 27 days after the last dose was administered to the mother. Statistically, only one tissue, the kidney, showed a significant difference ($P < 0{\cdot}001$) between male and female and this was at the higher dosage level.

The liver weights expressed as a precentage of body weights and the results of the microscopic examination are given in Table 4. There was a significant increase in the relative liver weights of dosed groups compared with those of controls.

The hepatocytes were enlarged in the majority of exposed rats. Vacuolization of the cytoplasm and proliferation of the bile ducts were seen in some rats. These morphological changes in the liver were more pronounced at the higher dosage.

Discussion

Components derived from Aroclor 1254 were found in the tissues of the foetuses taken from dosed mothers, but none were found in the control foetuses. This shows that transplacental passage of Aroclor occurs in rats. When 50 mg Aroclor/kg/day was given, only

Table 3. *Mean concentrations of PCB-derived components found in 21-day-old weanling rats from dams given an oral dose of Aroclor 1254 on days 7–15 of pregnancy*

PCB dose (mg/kg/day)	Levels of PCB-derived components (μg/g) in						Mean body weight of weanlings (g)	
	Brain		Liver		Kidney			
	Males	Females	Males	Females	Males	Females	Males	Females
0	< 0·74	< 0·94	< 0·19	< 0·22	< 0·36	< 0·30	42·4	38·5
10	2·16 ± 0·10	1·97 ± 0·43	3·35 ± 0·39	5·08 ± 1·64	1·69 ± 0·38	2·20 ± 0·24	34·8	33·4
50	7·69 ± 1·37	7·04 ± 1·91	18·24 ± 4·00	16·55 ± 2·79	6·99 ± 0·27*	2·92 ± 0·59	33·8	32·0

Values for tissue levels in the dosed group are means ± SEM for samples from three weanlings. That marked with an asterisk differs significantly (Student's *t* test) from the corresponding value for the other sex: $^*P < 0·001$. The *t* test showed no significant differences between the body weight in weanlings from the control and dosed groups.

Table 4. *Relative liver weights and morphology of livers from 21-day-old weanlings from dams given an oral dose of Aroclor 1254 on days 7–15 of pregnancy*

PCB dose (mg/kg/day)	Mean liver weight (% of body weight)		Liver morphology: Light microscopic findings in rats of both sexes
	Males	Females	
0	3·8 ± 0·12	3·7 ± 0·06	Normal
10	$4{\cdot}8 \pm 0{\cdot}12^{**}$	$5{\cdot}1 \pm 0{\cdot}09^{*}$	Slightly enlarged and vacuolated hepatocytes in 15/21 rats
50	$5{\cdot}9 \pm 0{\cdot}13^{*}$	$5{\cdot}8 \pm 0{\cdot}06^{*}$	Slightly enlarged hepatocytes in 15/20 rats; vacuolization of the cytoplasm of the liver cells in 10/20 rats; bile-duct proliferation surrounded by fibrosis in 5/20 rats

Values are means ± SEM for three weanlings of either sex and those marked with asterisks differ significantly (Student's *t* test) from the control values: $^{*}P < 0{\cdot}001$; $^{**}P < 0{\cdot}005$.

twice as much PCB was found in the foetus as when 10 mg/kg/day was given. It is possible that distribution of the PCBs between the dam and the foetus is not uniform and that a partial barrier exists. This needs further investigation.

The PCB levels observed in the weanling rats resulted at least in part from nursing. The milk concentration of PCBs substantiates this conclusion. Also, the morphological changes observed in the liver, due to PCBs, most probably result from the PCB levels in the milk. An indication of the difference contaminated milk can make was shown for Mirex by Gaines & Kimbrough (1970). Sucklings exposed to mirex-contaminated milk developed cataracts, yet most sucklings raised on mirex-free milk did not, although they had been exposed *in utero* to this compound.

A statistical comparison of the control and dosed groups, with respect to the total weight of litters and the percentage of foetuses born dead, showed no significant difference.

Although the changes in liver morphology were more consistent at the higher dose, the overall effects of Aroclor ingestion on the liver parallel some findings previously observed (Kimbrough, Linder & Gaines, 1972). Rat livers were not all affected equally by the same dose. This has been observed in our laboratory before in PCB studies. It may be due, in part, to variations among individual animals and/or to impurities that may be constituents of the Aroclor preparations (Vos, Koeman, van der Maas, ten Noever de Brauw & de Vos, 1970).

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Hormonal Influences on DDT Metabolism in the White Rat[1]

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ABSTRACT

The influence of diethylstilbestrol (DES) and testosterone propionate (TP) on the storage of DDT and its metabolite, DDE, was studied in normal and in gonadectomized rats. As expected, TP dosage increased the growth of female rats while DES administration inhibited the growth of males. Both hormones inhibited gonadal growth. Rats that received either DDT or hormone showed an increased liver weight/body weight ratio as compared to control animals. This effect was more marked in males than in females. DES increased DDT and DDE storage in fat in the male while TP decreased these values in the female. Similar effects of hormone dosage were noted on the ratio of DDE to total DDT-derived material stored in fat.

THE ACUTE ORAL TOXICITY to white rats of DDT dissolved in oil does not appear to be influenced by the sex of the animals tested (1). However, female rats are somewhat more susceptible to repeated doses of the insecticide than are male rats (2, 3). Sex differences are also apparent in liver histopathology and in fat storage. Male rats show much more frequent and extensive histological changes in the liver than female rats when both sexes are exposed repeatedly at moderate dosage levels (4). The fact that female rats store more DDT in their fat than male rats fed at the same dosage level is well established (5). The evidence for other species is not so clear. However, Harris *et al.* (6) found no difference between male and female pigs with respect to DDT storage. If a difference exists for monkeys or dogs, it is very small.

Burlington and Lindeman (7) showed that DDT produced a striking inhibition of testicular growth and secondary sexual characters of cockerels. They speculated that the DDT may have had an estrogen-like action, and pointed out that the structural formulae of DDT and diethylstilbestrol were similar. Fisher *et al.* (8) demonstrated conclusively that DDT has no estrogenic activity in ovariectomized rats, although an analog [2, 2'-bis-(*p*-hydroxyphenyl)-1, 1, 1-trichloroethane] did show such activity in conformity with an hypothesis on the chemical nature of estrogens.

The present study was designed to investigate the physiological basis of the marked sex difference which rats show with respect to fat storage of DDT following repeated oral doses. Dubois *et al.* (9) have reported that administration of a male sex hormone to female rats and of a female hormone to male rats tended to equalize their susceptibility to a single dose of parathion. In the present experiment, the influence of diethylstilbestrol (DES) and testosterone propionate (TP) on the storage of DDT and its metabolite, DDE, has been studied in normal and in gonadectomized rats.

METHODS

One hundred male and eighty female weanling white rats of the Sherman strain were used in the study. They were divided into groups of 10 rats each. All the rats within a group were placed on identical treatment schedules. The treatment and identity of these groups are outlined in table 1.

The animals receiving DDT were given this insecticide admixed into their regular diet of ground Purina laboratory chow at a concentration of 200 parts per million (ppm) for a 120-day period. Pure, recrystallized DDT was used. DES and TP were given subcutaneously in peanut oil solution three times per week at the daily rates of 0.4 and 0.8 mg/kg, respectively. One group was given peanut oil only, using the same volume as was required in administering the hormones. Those rats receiving both medications were

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TABLE 1. WEIGHT OF GONADS AND LIVER OF RATS STUDIED FOR DDT AND DDE STORAGE

Group, Sex	Diet	Hormone Dosage	Av. Body Wt.	Gonad Wt/ Body Wt. Ratio	Liver Wt/ Body Wt. Ratio
			gm	%	%
1, ♂	Chow	None	400	0.77±0.03†	2.70±0.15†
2, ♂	Chow	DES	210	0.15±0.03	4.34±0.09
3, ♂	DDT	None	363	0.83±0.30	3.47±0.08
4, ♂	DDT	DES	224	0.13±0.01	4.99±0.13
5,* ♂	DDT	None	320		3.19±0.03
6,* ♂	DDT	DES	220		5.12±0.14
7, ♀	Chow	None	252	0.04±0.001	2.80±0.07
8, ♀	Chow	TP	289	0.02±0.002	3.07±0.14
9, ♀	DDT	None‡	229	0.04±0.002	3.57±0.05
10, ♀	DDT	None	225	0.04±0.001	3.45±0.09
11, ♀	DDT	TP	257	0.02±0.002	3.64±0.10
12,* ♀	DDT	None	282		3.18±0.02
13,* ♀	DDT	TP	271		3.28±0.03

* Rats in *groups 5, 6, 12* and *13* were gonadectomized.
† Standard error of the mean. ‡ These rats received peanut oil only.

given the hormone for 1 week prior to initiation of DDT feeding and they then continued to receive hormone and DDT concurrently until they were killed. Gonadectomies were performed on some groups of rats 10 days before any medication was given.

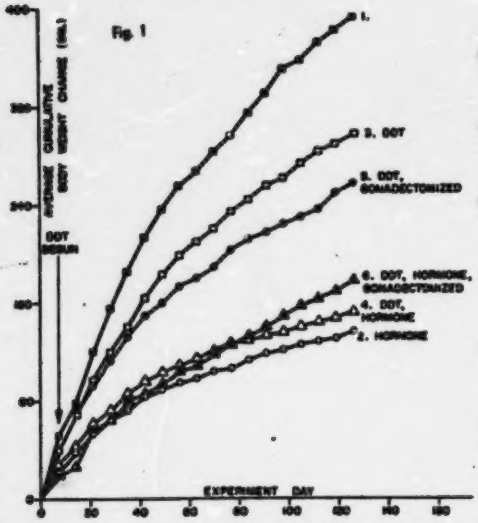
All the rats were killed by an overdose of chloroform vapor at the end of the dosage period, and samples of perirenal fat were taken for analysis of DDT and DDE content using a modification of the Schechter-Haller procedure (10). Liver and gonad weights were also recorded at autopsy.

RESULTS

Growth. A comparison of figures 1 and 2 shows that the male rats receiving DES gained weight at essentially the same rate as female rats and, thus, at a much slower rate than did normal males. Castration brought about a lesser diminution in weight gain. Castration did not seem to potentiate the hormonal action on growth since the weight gains in the intact animals receiving DES and in the castrated rats receiving this estrogen were essentially similar. Normal male rats that received 200 ppm DDT in their chow showed a somewhat smaller weight gain than those fed plain chow.

The growth curves of the female rats are shown in figure 2. Administration of male sex hormone stimulated the growth of both normal and DDT-fed female rats, but had a slight inhibitory effect on the weight gain of the spayed animals. Ovariectomy appeared to have about the same degree of growth-stimulating effect as hormone administration. DDT significantly inhibited the growth of all groups to which it was fed.

Sexual Development. The testis and ovary weights are given in table 1. This table shows that the sex hormones had a marked inhibitory effect on gonadal growth when given to rats



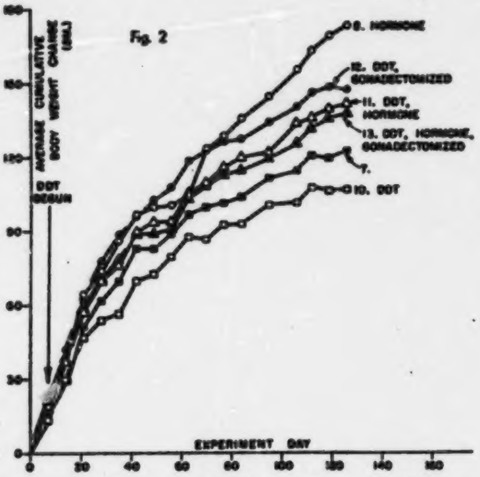


FIG. 1. Average cumulative body weight changes in groups of male rats studied for storage of DDT and DDE in fat. Group numbers (table 1) and positive treatment are indicated.

FIG. 2. Average cumulative body weight changes in groups of female rats studied for storage of DDT and DDE in fat. Group numbers (table 1) and positive treatment are indicated.

of the opposite sex. No significant effects of DDT on gonadal weight were noted.

Liver Weight. The significance of variations in liver weight can best be judged on the basis of the liver weight/body weight ratio. This value is shown in table 1.

The animals that received either hormone alone or with DDT showed in every instance an increased liver weight ratio as compared to their respective controls. Animals receiving DDT alone, or DDT and a hormone, showed a higher liver weight/body weight ratio than controls fed plain chow. These differences were present in both males and females, but were more marked in the former.

The peanut oil vehicle alone was investigated in DDT-fed rats (*group 9*) and was found to have no significant additional effect on liver weight.

DDT Storage. Figure 3 shows that administration of DES to males increased DDT storage, whereas administration of TP to females decreased DDT storage.

Castration also increased DDT storage in the male rats, although the magnitude of the effect was less than with DES administration. In the case of the castrated rats, somewhat less DDT was detected in the fat of the hormone-dosed animals. The normal female rats stored over twice as much DDT as the highest male group, namely intact males which had received DDT and hormone.

Gonadectomized female rats showed somewhat less modification of their DDT storage pattern than did intact animals dosed with hormone. However, in contrast to the finding with males, there appeared to be an additive effect between spaying and hormone administration in the female, since the group which received both treatments showed a greater diminution of their DDT storage level than the groups which received either treatment alone.

Dosage with the hormone of the opposite sex reduced but did not quite eliminate the difference between the sexes with respect to DDT storage.

DDE Storage. In the case of both the unoperated and the castrated male rats the DES-dosed groups stored, on the average, slightly more DDE than did their respective controls. The hormone potentiated DDE storage in the male to a greater extent than did castration. There was no additive effect between the two treatments in this sex.

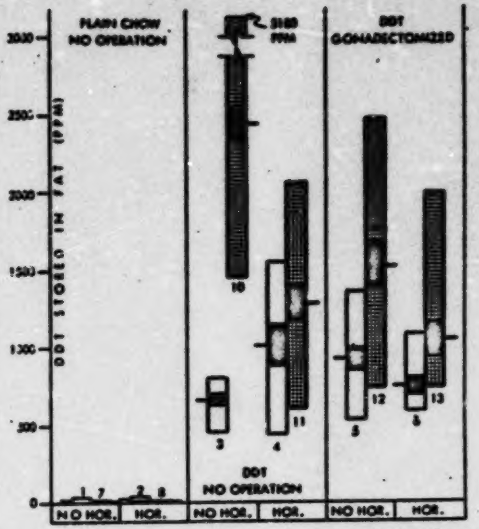


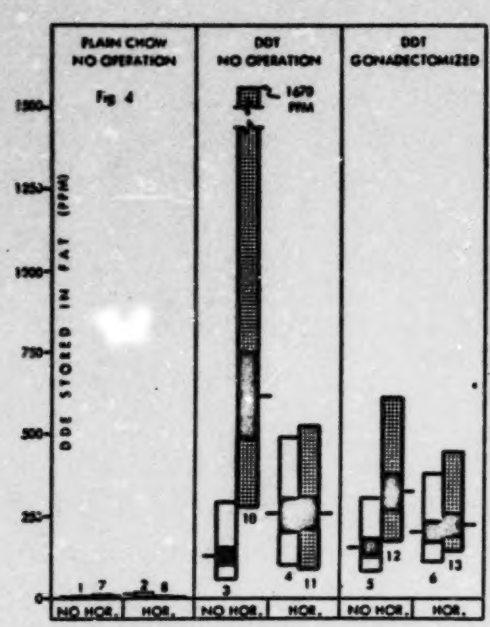
FIG. 3. DDT storage in fat for groups of rats given indicated treatment. *Horizontal line* indicates the group mean, *checkered area* encompasses one standard error on each side of the mean, and the entire *enclosed area* covers the range of values. *Clear bars* represent males and *shaded bars* represent females.

The administration of TP to female rats brought about a decrease in DDE storage both in the intact and in the spayed animals. The hormone was more effective than ovariectomy in this regard. There was some indication of an additive effect between the two treatments.

Dosage with sex hormones eliminated the sex difference with respect to DDE storage. These results are shown in figure 4.

Ratio of DDE to Total DDT-Derived Material Stored in Fat. The fractional part of the DDT-derived material stored in fat as DDE for the various groups of rats studied is shown in figure 5. As expected, the groups that were fed plain chow converted a higher proportion of their small DDT intake to DDE than did the groups fed larger DDT dosages.

The DES-dosed male rats showed a higher percentage of DDE than did the controls. This was true of the unoperated, of the castrated DDT-fed rats, and of those fed plain chow. All three of these groups of estrogen-dosed male rats showed about the same or somewhat



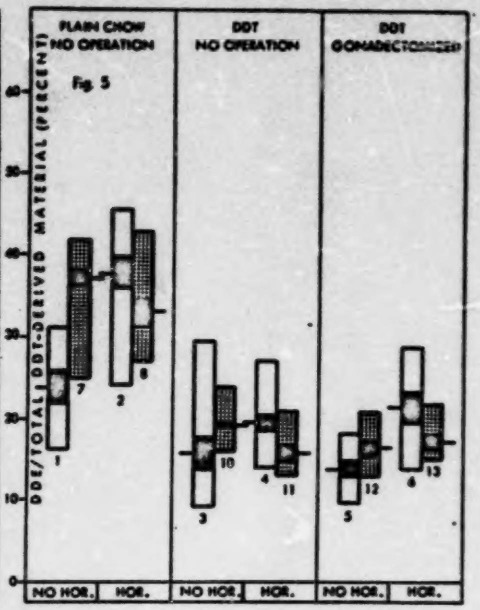


FIG. 4. DDE storage in fat for groups of rats given indicated treatment. Other conventions are as in fig. 3.

FIG. 5. Average ratio of DDE to total DDT-derived material stored in fat for groups of rats studied for DDT and DDE storage in fat. Other conventions are as in fig. 3.

higher DDE percentages than did comparable female rats.

The male sex hormone decreased the percentage of DDE in female rats fed plain chow and in unoperated female rats fed DDT. However, no effect of this treatment was noted in spayed females.

DISCUSSION

Results similar in all respects to those discussed above were obtained in a separate preliminary trial run at a different time. In this test only three groups of male rats were studied: those on a normal diet given estrogen, and DES-dosed and sexually-normal male rats fed on a diet containing DDT.

The hormone dosage given the rats was apparently adequate as indicated by its almost complete inhibition of gonadal development and by its effect on growth.

Statistical analysis based on Student's *t* test (11) has been applied to the results of the tests concerned with the effect of hormone dosage on DDT and DDE storage in fat and on the ratio of DDE to total DDT-derived material. For the purpose of this statistical analysis, all the animals (both intact and gonadectomized) given hormone were considered together.

TABLE 2. STATISTICAL SIGNIFICANCE OF HORMONAL EFFECTS ON FAT STORAGE OF DDT AND DDE BY RATS

Hormone Dosage	No. of Animals	DDT Storage in Fat	DDE Storage in Fat	DDE/Total DDT-Derived Material Stored in Fat
		ppm	*ppm*	%
		Males		
None	28	771 ± 42	114 ± 14	12.3 ± 1.1
DSB	29	920 ± 49*	222 ± 11†	19.0 ± 0.8†
		Females		
None	30	2072 ± 151	503 ± 51	19.2 ± 0.7
TP	20	1163 ± 102†	241 ± 30†	16.6 ± 0.6*

Values given are mean ± standard error.

* *P* for difference <0.05. †*P* for difference <0.01.

The differences between normal male and normal female rats with respect to DDT and DDE storage in fat and to DDE ratio are significant at the 99% level. The effect of hormone administration was significant at the 95% level, or higher, in both sexes with respect to DDT storage, DDE storage and DDE ratio. These results are shown in table 2.

The high significance levels (table 2) for the effect of sex hormones on storage of DDT and DDE in fat indicates that an endocrine mechanism may account for the sex differences in this regard.

The possibility exists that the pituitary gland may play a role. However, the results make this appear unlikely, for, although both male and female rats have their pituitary activity inhibited by exogenous doses of sex hormone, the effect on their storage of DDT and DDE is opposite. Were the pituitary primarily involved, a decrease in its activity should presumably shift the storage equilibrium in the same direction in both sexes.

In view of the ubiquity among mammals of sex hormones, the basic reason for the uniqueness of rats with respect to sex differences in DDT and DDE storage is still unexplained.

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BODY BURDEN OF PESTICIDES IN MAN

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(deals)

The concept of storage or body burden is more important in relation to the chlorinated hydrocarbon insecticides than to other groups of synthetic pesticides because of the relatively greater persistence of the former in human tissues. For this reason, the present paper will deal primarily with the chlorinated hydrocarbon insecticides, although limited consideration will also be given to the organic phosphorus and carbamate compounds.

The chlorinated hydrocarbon insecticides and many of their metabolites are stored in adipose tissue in exposed animals. This fact is not surprising in view of the known solubility of these compounds in fats and fat solvents and their practical insolubility in aqueous solvents. However, the quantitative differences in storage among these compounds do not parallel the differences in their solubility in fat. For example, in studies with rats, Sedlak (1965) noted that the equilibrium storage levels of BHC isomers following dietary intake were unrelated to the solubility of the compounds in rat fat, with the exception that solubility was a limiting factor.

Among the factors that may influence storage are compound, intensity and duration of dosage, efficiency of absorption, species, age, sex, nutritional status, and the integrity of organs, especially the liver and kidneys (Hayes, 1965). Recent work with experimental animals has shown that storage levels of some chlorinated hydrocarbon insecticides can also be modified by previous or concurrent doses of drugs (Cueto & Hayes, 1965; Street *et al.*, 1966) or other pesticides (Street, 1964).

It is a fundamental axiom in pharmacology that all compounds reach, or at least approach, a steady state of storage, in which the daily dosage and the daily excretion are equal, provided the dosage is constant and is tolerated by the organism. FIGURE 1 shows the increase of DDE storage in body fat in rats with continued dosage, the eventual attainment of a steady state, and the relatively slow loss of the compound from storage. For a given compound and species, there will be a different plateau level of storage for each dosage. In general, doubling the dosage will double the storage, but in some instances storage may not increase quite as rapidly as dosage. Apparently, the major factor that accounts for the occurrence of this plateau with regard to storage of the chlorinated hydrocarbon pesticides is the fact that, in the excretion of these compounds, a constant proportion of the stored material is lost per unit time; thus, the daily excretion of DDT, for example, is greater when the storage is high than when it is low. As an analogy, one might consider a hydraulic example, in which water flows into a bucket from a faucet (dosage) and out through a hole in the bottom (excretion). In this example, the rate of water outflow through the hole varies with the depth of water in the bucket, being more rapid when the water level is high and slower when the level is low. It is evident that, at a constant inflow (dosage), one will eventually reach a water level in which inflow and outflow are exactly balanced. This corresponds to the steady state or plateau level.

An additional factor that may play some role in the attainment of the storage level plateau is the induction of microsomal enzymes. It is known that continued exposure to low levels of a number of the chlorinated hydrocarbon insecticides produces an increase in the activity level of certain drug-metabolizing enzymes in the liver (Durham, 1967). It would seem entirely possible that continued exposure to a specific pesticide could stimulate the enzyme system that metabolizes that pesticide.

The compound that has been studied most carefully in regard to storage in man is DDT. The earliest published report of DDT storage in man is apparently that of Howell (1948). He found DDT-derived material at a concentration of about 7 ppm in the fat of a man with occupational exposure for four years and a history of eating foods known to bear appreciable residues. The first general survey for DDT was carried out by Laug and his colleagues in California in 1950 (Laug *et al.*, 1951). They demonstrated DDT residues ranging from 0 to 34 ppm (mean, 5.3 ppm) in the fat of 75 persons with no special occupational exposure. The results of this and subsequent surveys of the concentration of DDT and its metabolites in body fat in the general population of the United States are summarized in TABLE 1. It is important at this point to note that fat samples from people who died before DDT was manufactured did not reveal DDT or DDT-like materials on analysis with a colorimetric method (Hayes *et al.*, 1958).

Since the 1950 study by Laug *et al.*, 1951, a total of 9 separate surveys involving a total of 717 subjects has been conducted in various areas of the country to define the DDT storage level more exactly and to study its changes with time and geographic location. The results show that DDT is found in practically all persons in the general population of the United States. The present mean storage level is about 3 ppm for DDT and about 8 ppm expressed as DDT equivalent for metabolites of this compound. However, there has been no significant increase in the storage of DDT by the general population of the United States since this level was

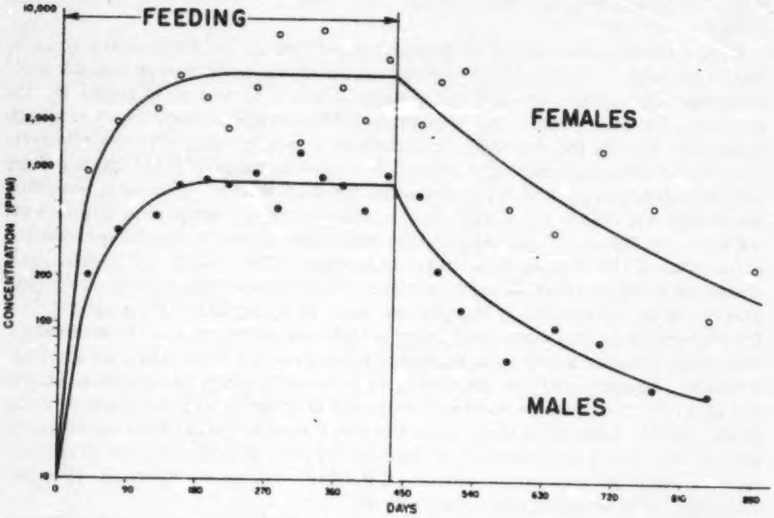

FIGURE 1. Storage of DDE in body fat of male and female rats during and after feeding on a diet containing 200 ppm DDE.

TABLE 1

CONCENTRATION OF DDT-DERIVED MATERIAL IN BODY FAT OF THE GENERAL POPULATION OF THE UNITED STATES

Year	Location	No. of Samples	Analytic Method	DDT (ppm)	DDE as DDT (ppm)	Total as DDT (ppm)	DDE as DDT (% of total)	Reference
1942	Louisville, Ky.	10	colorimetric	*	*	*	—	Hayes *et al.*, 1958
1950	California	75	colorimetric	5.3	—	5.3	—	Laug *et al.*, 1951
1955	Tallahassee, Fla.	49	colorimetric	7.4	12.5	19.9	63	Hayes *et al.*, 1956
1954–1956	Savannah, Ga. and Wenatchee, Wash.	61	colorimetric	4.9	6.8	11.7	58	Hayes *et al.*, 1958
1956	Atlanta, Ga.	36	colorimetric	5.5	10.1	15.6	65	Hayes *et al.*, in preparation
1961–1962	Atlanta, Ga., Louisville, Ky., Phoenix, Ariz. and Wenatchee, Wash.	130	colorimetric	4.0	8.7	12.7	69	Quinby *et al.*, 1965a
1961–1962	Wenatchee, Wash.	28†	GLC‡	2.4	4.3	6.7	64	Dale & Quinby, 1963
1962–1963	Chicago, Ill.	282	GLC‡	2.9	8.2	11.1	74	Hoffman *et al.*, 1964
1964	Northeast, Midwest, Deep South, and Far West	64	GLC‡	2.5	5.1	7.6	67	Zavon *et al.*, 1965
1964	New Orleans, La.	25	GLC‡	2.3	8.0	10.3	77	Hayes *et al.*, 1965

* Not detected.

† These 28 samples also tested for DDT and DDE content by a chlorimetric method are included in the 130 samples listed directly above.

‡ Gas-liquid chromatography.

first measured in 1950. Studies in volunteers have shown that the excretion of DDT is very slow in man (Hayes *et al.*, manuscript in preparation). Thus, any decrease in storage of DDT as the result of decrease of DDT levels in food or other environmental media would be reflected only slowly in decreased fat storage levels of the compound.

Since the 1950 survey by Laug *et al.* (1951), there have been many improvements in the analytical chemical methods available for measuring DDT and related compounds. The first improvement, introduced by Mattson *et al.* (1953), permitted the measurement of DDE through an adaptation of the classic Schecter-Haller colorimetric method. DDE, which is one of the chief metabolites of DDT, had been entirely missed by earlier analytical methods. However, by suitable procedures the concentration of this metabolite in the fat of the general population was shown to be higher than that of the parent compound. Later, the introduction of gas liquid chromatography permitted chemists to distinguish the various stereoisomers of DDT and DDE and to detect the analog, DDD.

The general population levels of DDT storage have also been determined in a number of other countries, including Canada, Czechoslovakia, Denmark, England, France, Hungary, India, Israel, Italy, and West Germany. The results of these studies are shown in TABLE 2. It is interesting to note that the levels of DDT in many of these other countries are lower than those in the United States, although the levels in Czechoslovakia, Hungary, India, and Israel are as high as or higher than the American values.

The storage of DDT in people with environmental, occupational, or other special exposure to the insecticide is summarized in TABLE 3. People with environmental exposure–defined as living within 500 feet of a DDT-treated agricultural area–showed little difference in DDT storage from the general population. However, meat abstainers and eskimos, whose diet was relatively free of DDT, stored significantly less of the compound than did the general population. On the other hand, people with occupational exposure to the compound stored significantly higher levels in proportion to the magnitude of their exposure. Apparently, the highest recorded storage in a healthy man was 648 ppm measured in the fat of a formulator. Storage levels for volunteers given known daily doses of DDT at dosages as high as 35 mg reached mean concentrations of 281 ppm after 21.5 months of dosage. The men maintained apparent good health.

As shown in FIGURE 2, there is a direct relationship between DDT dosage and DDT storage in man. The higher curve on this figure presents the intake (0.038 mg/day)/storage (3.9 ppm) correlation for the most recent studies in our own laboratories (Durham *et al.*, 1965; Quinby *et al.*, 1965a) and for other studies in which these quantities have been determined. Meat abstainers had a mean daily DDT intake of 0.041 mg and showed an average DDT storage level of 2.3 ppm (Hayes *et al.*, 1958). The daily dietary DDT intake of 0.164 mg for the general population of this country in 1954 (Walker *et al.*, 1954) determined a DDT storage level of 4.9 ppm (Hayes *et al.*, 1958). In a study of prisoner volunteers, the mean daily dietary intake of DDT was found to be 0.202 mg, and the DDT storage level was 7.4 ppm (Hayes *et al.*, 1956). The higher points on these curves represent values from a study in which volunteers were fed known amounts of DDT (3.5 and 35.0 mg/man/day); among other observations, their storage of DDT and DDE was measured (Hayes *et al.*, 1956; Hayes *et al.*, to be published). The lower curve on this figure presents similar data on the relationship between dosage and storage for dieldrin in man.

The closeness of fit on both the DDT and dieldrin intake/storage curve (FIGURE

TABLE 2

CONCENTRATION OF DDT-DERIVED MATERIAL IN BODY FAT OF THE GENERAL POPULATION OF COUNTRIES OTHER THAN THE UNITED STATES

Country	Year	No. of Samples	Analytic Method	DDT (ppm)	DDE as DDT (ppm)	Total as DDT (ppm)	DDE as DDT (% of total)	Reference
Canada	1959–1960	62	colorimetric	1.6	3.3	4.9	67	Read & McKinley, 1961
Czechoslovakia	1963–1964	229	colorimetric	5.5	4.1	9.6	43	Halacka *et al.*, 1965
Denmark	1965	18	GLC*	0.6	2.7	3.3	82	Weihe, 1966
England	1961–1962	131	colorimetric	—	—	2.2	—	Hunter *et al.*, 1963
England	1963–1964	66	GLC*	1.1	2.2	3.3	67	Egan *et al.*, 1965
England	1964	100	GLC*	—	—	3.9	—	Robinson *et al.*, 1965
France	1961	10	colorimetric	1.7	3.5	5.2	67	Hayes *et al.*, 1963
Hungary	1960	48	colorimetric	5.7	6.7	12.4	48	Dénes, 1962
India (Delhi area, civilian)	1964	67	colorimetric	16	10	26	39	Dale *et al.*, 1965
India (other cities, military)	1964	16	colorimetric	8	5	13	37	Dale *et al.*, 1965
Israel	1963–1964	254	colorimetric	8.5	10.7	19.2	56	Wassermann *et al.*, 1965
Italy	1965	9	GLC*	1.8	3.2	5.0	64	Kanitz & Castello, 1966;
West Germany	1958–1959	60	colorimetric	1.0	1.3	2.3	57	Maier-Bode, 1960

* Gas-liquid chromatography.

TABLE 3

CONCENTRATION OF DDT-DERIVED MATERIAL IN BODY FAT OF PEOPLE IN THE UNITED STATES WITH SPECIAL EXPOSURE (ENVIRONMENTAL, OCCUPATIONAL, OR DIETARY) TO DDT[*]

Exposure	Year	No. of Samples	DDT (ppm)	DDE as DDT (ppm)	Total as DDT (ppm)	DDE as DDT (% of total)	Reference
Died before DDT	<1942	10	†	†	†	—	Hayes *et al*, 1958
Meat abstainers	1955–1956	16	2.3	3.6	5.9	61	Hayes *et al*, 1958
Eskimos[‡]	1960	20	0.8	2.2	3.0	73	Durham *et al*., 1961
Environmental[§]	1954–1956	110	6.0	9.6	15.6	62	Hayes *et al*, 1958
Environmental[§]	1961–1962	28	4.3	8.6	12.9	67	Quinby *et al*., 1965a
Applicators	1954–1956	30	14.0	21.1	35.1	60	Hayes *et al*., 1958
Applicators	1961–1962	14	10.7	24.1	34.8	69	Quinby *et al*., 1965a
Formulator	1951	1	122	141	263	54	Mattson *et al*., 1953
Formulator	1954	1	648	483	1131	43	Hayes *et al*., 1956
Volunteers given 3.5 mg per day orally[¶]	1953–1954	2	30	3.9	34	11	Hayes *et al*., 1956
Volunteers given 3.5 mg per day orally[¶]	1957–1958	6	50	21	71	30	Hayes *et al*., in preparation
Volunteers given 35 mg per day orally[¶]	1953–1954	6	234	24	258	9	Hayes *et al*., 1956
Volunteers given 35 mg per day orally[¶]	1957–1958	6	281	40	321	12	Hayes *et al*., in preparation

[*] All analyses were done using a colorimetric method.

[†] Not detected.

[‡] Alaskan eskimos who ate predominately a native diet shown to contain little or no DDT.

[§] Residents of the Wenatchee, Wash. area living within 500 ft of DDT-treated agricultural area.

[¶] Based on samples taken after 11 months or more of dosage.

[**] Based on samples taken after 21.5 months of dosage.

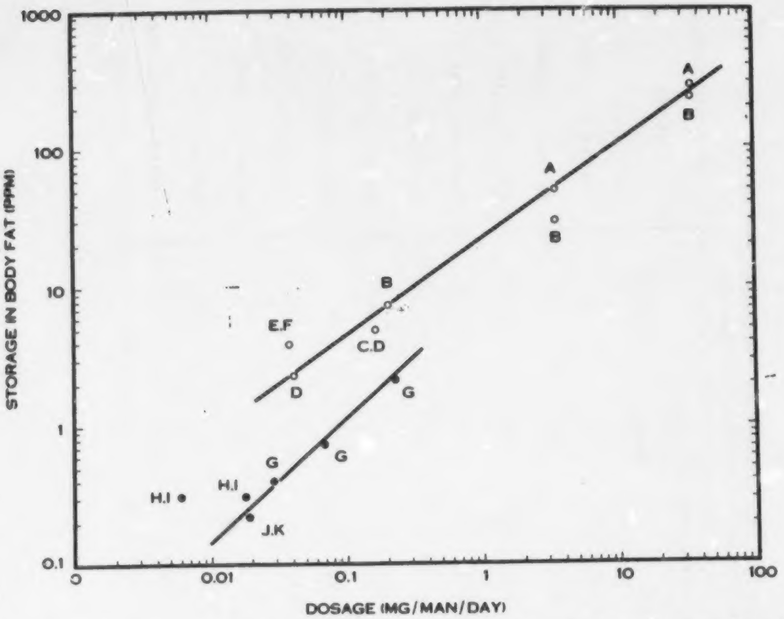

FIGURE 2. Storage of DDT (*upper line*) and dieldrin (*lower line*) in the body fat of people in relation to dosage. The letters beside the points refer to references as follows: **A.** Hayes *et al.*, to be published. **B.** Hayes *et al.*, 1956. **C.** Hayes *et al.*, 1958. **D.** Walker *et al.*, 1954. **E.** Quinby *et al.*, 1965a. **F.** Durham *et al.*, 1965. **G.** Hunter and Robinson, to be published. **H.** Zavon *et al.*, 1965. **I.** Dugan *et al.*, 1966 (values for daily intake plotted for dieldrin alone and for dieldrin plus aldrin). **J.** Robinson & Hunter, 1966. **K.** Robinson and McGill, 1966.

2) of the points whose dosage aspects are determined solely by dietary intake of the pesticide would seem to indicate that this source accounts for the greater part of the exposure to these compounds of the general population in this country. Furthermore, the concentrations of pesticides in potable water are extremely low (Faust & Aly, 1964; Durham, 1965) and significant quantities of pesticides have not been reported in the air, except in work areas. These facts confirm the conclusion reached independently by Campbell and his colleagues that food contributes over 90 per cent of the DDT absorbed by people in the general population (Campbell *et al.*, 1965).

Gas liquid chromatography has also made possible the measurement of the small traces of other chlorinated hydrocarbon insecticides, in addition to DDT and its metabolites, that are stored in human fat. A summary of these results for the general population of this and other countries is shown in TABLE 4. It can be seen that people in the United States and other countries store BHC (0.02 – 1.43 ppm), dieldrin (0.04 – 0.59 ppm), and heptachlor epoxide (up to 0.24 ppm). The presence of endrin in fat samples from the general population was suggested in gas chromatographic tracings obtained by other investigators, but the levels were quite low, and definite confirmation was lacking. However, Kanitz and Costello (1966) reported 0.93 ppm of endrin and 0.18 ppm of aldrin from the

TABLE 4

CONCENTRATION OF CHLORINATED HYDROCARBON PESTICIDES IN BODY FAT OF THE GENERAL POPULATION OF VARIOUS COUNTRIES AS DETERMINED BY GAS-LIQUID CHROMATOGRAPHY

Country	Year	No. of Samples	Storage Level in Body Fat (ppm)				Reference
			BHC Isomers	Dieldrin	Endrin	Heptachlor Epoxide	
United States	1961–1962	28	0.20	0.15	—	—	Dale & Quinby, 1963
United States	1962–1963	282	0.57	0.11*	nd	—	Hoffman *et al.*, 1964
United States	1964	64	—	0.31	<0.02	0.10	Zavon *et al.*, 1965
United States	1964	25	0.60	0.29	<0.03	0.24	Hayes *et al.*, 1965
Denmark	1965	18	—	0.20	—	—	Weihe, 1966
England	1961–1962	131	—	0.21	—	—	Hunter *et al.*, 1963
England	1963–1964	65	0.42	0.26	—	<0.1	Egan *et al.*, 1965
England	1964	100	0.02†	0.21	<0.02	<0.01	Robinson *et al.*, 1965
France	1961	10	1.19	—	—	—	Hayes *et al.*, 1963
India	1964	35	1.43	0.04	nd	nd	Dale *et al.*, 1965
Italy	1965	9	—	0.59‡	0.93	—	Kanitz & Castello, 1966

nd = not done.

* Only 64 samples were tested for dieldrin content.

† Only 20 samples were tested for BHC content.

‡ These authors reported 0.18 ppm of aldrin.

general population in Italy. Both of these findings are unexpected—the endrin level because of its magnitude and the aldrin because of its presence in any amount. Generally, aldrin is converted to dieldrin and stored as dieldrin in the animal body. Also, it is possible that at least a part of the stored DDD detected in human tissues resulted from insecticidal use of the compound, although it has been shown that DDD is a metabolite of DDT in certain species.

These other compounds, with the possible exception of dieldrin, have not been measured in man over a significant length of time to determine whether their storage has reached a steady state, as has that for DDT. In England the levels of dieldrin have not changed appreciably in either the fat (Robinson *et al.*, 1965) or the blood (Robinson & Hunter, 1966) of the general population since 1961 and 1962, respectively.

To investigate the possibility of an etiologic relationship, some studies have been made on storage of chlorinated hydrocarbon pesticides in persons with various diseases. For example, Maier-Bode (1960) found no essential difference in storage of DDT or DDE between 21 persons who died of cancer and 39 persons who died of other diseases. Robinson *et al.* (1965) detected no differences in total DDT-derived material or dieldrin between 50 biopsy and 50 necropsy samples. Neither was there any correlation for the necropsy samples between storage level and cause of death classified as neoplasm, cardiovascular disease, infection, or accident. Quinby *et al.* (1965a) could detect no differences in DDT or DDE levels between smokers and nonsmokers.

In studies in rats, it has been shown that mobilization of body fat during starvation resulted in an increased concentration of DDT-derived material in that tissue and a corresponding increase in plasma, liver, kidney, and brain (Dale *et al.*, 1962). The results of this work and of another study of single doses of DDT in the rat showed that clinical signs of poisoning were correlated directly with the concentration of DDT in the nervous system, as indicated by the concentration in the brain (Dale *et al.*, 1963).

Methods are now available for measuring the chlorinated hydrocarbon insecticides and their metabolites in human blood. Some results obtained using these procedures are summarized in TABLE 5. It can be seen that, in the general population of the United States, the concentrations ranged from a low of 0.0008 ppm for heptachlor epoxide to a high of 0.0114 ppm for DDE.

It is interesting to consider the relationship between the concentrations of the chlorinated hydrocarbon insecticides in blood and in depot fat. In a study of 44 persons from the general population in England, Robinson and Hunter (1966) found mean blood levels of 0.0130 ppm for DDT and 0.0014 ppm for dieldrin and depot fat levels of 4.0 ppm for DDT and 0.22 ppm for dieldrin. These values determined fat/blood ratios of 306 for DDT and 159 for dieldrin. These authors concluded that the lower fat/blood ratio for dieldrin indicated that a greater proportion of the total body burden of dieldrin was present in the blood, in comparison with DDT.

DDT has been found in human milk in the United States (Laug *et al.*, 1951; West, 1964; Quinby *et al.*, 1965b), Hungary (Denes, 1964), and England (Egan *et al.*, 1965). The mean levels in this country ranged from 0.08 to 0.13 ppm in whole milk. These amounts correspond to about 2.0 to 3.3 ppm in the lipid fraction. Only the English samples reported by Egan and his co-workers (1965) were analyzed by gas chromatography and tested for pesticides other than DDT and its congeners. BHC (0.013 ppm) and dieldrin (0.006 ppm) were found.

The relatively low fat content of human organs with the correspondingly low

TABLE 5

CONCENTRATION OF CHLORINATED HYDROCARBON PESTICIDES IN BLOOD

Exposure	Year	No. of Samples	Type of Sample	Concentration (ppm)							Reference
				DDT	DDE	DDD	Total DDT Derived	BHC	Dieldrin	Heptachlor Epoxide	
General population, male, U.S.	1965	10	Whole blood	0.0068	0.0114	—	0.0193	0.0031	0.0014	0.0008	Dale *et al.*, 1966a
General population, male, U.S.	1965	10	Plasma	0.0132	0.0257	—	0.0415	0.0034	0.0019	0.0011	Dale *et al.*, 1966a
General population, female, U.S.	1965	10	Serum	0.0093	0.0152	—	0.0260	0.0042	0.0013	0.0008	Dale *et al.*, 1966b
General population, England	1964	44	Whole blood	—	—	—	0.013	—	0.0014	—	Robinson & Hunter, 1966
Occupational	1964	136	Whole blood	—	—	—	—	—	0.0463	—	Robinson & Hunter, 1966

storage levels of chlorinated hydrocarbon pesticides make detection in these tissues difficult. However, as early as 1958 Hayes and his co-workers, using a colorimetric method, detected DDT in adrenal glands from 11 persons at a mean level of 3.4 ppm in extractable lipid–about 0.7 ppm on a whole-tissue basis. It is interesting to note that periadrenal fat taken from 9 of these 11 subjects showed a mean DDT level of 7.5 ppm in the lipid fraction. Radomski and Fiserova-Bergerova (1965) have recently reported DDT, DDE, and dieldrin in samples of brain, kidney, liver, and gonads from unselected subjects.

Studies on fat taken from various depots throughout the body have indicated that the storage level for DDT tended to be constant in depot fat from any location (Hayes *et al.*, 1958). The concentrations of DDT in various whole tissues and in corresponding lipid extracts for the general population of the United States are summarized in TABLE 6. The important role of lipid in the storage of the chlorinated hydrocarbon pesticides and the apparent correlation between fat content of organs and their level of these pesticides seem to emphasize the desirability in future studies of determining concentrations based both on total organ weight and on extractable lipid.

The organic phosphorus and carbamate insecticides are, in general, much less persistent in the environment and in the animal body than are the chlorinated hydrocarbon materials. Although the former two groups of compounds do not seem to be stored to any significant extent, their effects continue and may be cumulative insofar as the inhibition of cholinesterase continues. The combination between the organic phosphorus moiety and cholinesterase is generally more stable than that between the carbamate molecule and the enzyme. In fact, the carbamate-to-cholinesterase bond is so unstable that it is often difficult to carry out a meaningful test on blood from persons exposed to carbamates (Witter, 1963).

In the case of some organic phosphorus compounds, notably parathion and malathion, measurement of a metabolite in the urine has proved to be a more sensitive test of absorption than is measurement of blood cholinesterase (Arterberry *et al.*, 1961; Hayes *et al.*, 1961). Exposure to the carbamate compound carbaryl can also be measured by excretion of the metabolite alphanaphthol (Best & Murray, 1962).

As discussed in detail in the preceding sections of this paper, the sensitive modern techniques of analytical chemistry, particularly gas chromatography, have made possible the detection of minute residues of various pesticides or their metabolites or both, in human tissues. It should not be assumed that the finding of

TABLE 6

CONCENTRATION OF DDT IN VARIOUS WHOLE TISSUES AND CORRESPONDING LIPID FRACTIONS FROM THE GENERAL POPULATION OF THE UNITED STATES

Tissue	No. of Samples	DDT in Whole Tissue (ppm)	Lipid Content (%)	DDT in Lipid Fraction(ppm)	Reference
Adipose	130	4.0	59	6.8	Quinby *et al.*, 1965a
Blood	10	0.0068	(0.6)*	(1.1)†	Dale *et al.*, 1966a
Milk	10	0.06	3.26	1.8	Quinby *et al.*, 1965b
Adrenal	11	—	—	3.4	Hayes *et al.*, 1958

* This value for lipid content of human blood was not determined for these particular samples, but is a general population mean value (Sunderman & Boerner, 1949).

† Calculated by the reviewer.

detectable residues of these chemicals in human tissue is in itself evidence of a harmful effect. Rather, at this time it is important that careful clinical and pharmacologic observations, along with measurement of body burden of pesticides, be made in exposed workers and in poisoning cases. Such data are essential to a meaningful evaluation of the significance to public health of the levels of these compounds found in tissues and excreta of the general population.

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DDT Metabolism: Oxidation of the Metabolite 2,2-bis(p-Chlorophenyl)ethanol by Alcohol Dehydrogenase

Abstract. *A metabolite of DDT, 2,2-bis(*p*-chlorophenyl)ethanol, is a substrate of crystalline liver alcohol dehydrogenase. The oxidation of the substrate was detected spectrophotometrically. The* p*-nitrophenylhydrazone derivative of the product, 2,2-bis(*p*-chlorophenyl)acetaldehyde, was identified by comparing its mass spectrum and thin-layer chromatographic behavior with that of an authentic sample.*

The metabolism of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] in mammals gives rise to 2,2-bis(*p*-chlorophenyl)ethanol (DDOH) and 2,2-bis(*p*-chlorophenyl) acetic acid (DDA) (*1*). An intermediate aldehyde has been proposed but has not yet been found to occur in vivo. We have synthesized the proposed intermediate, 2,2-bis(*p*-chlorophenyl)acetaldehyde (DDCHO) (*2*).

Because the aldehyde has never been found in vivo and because preliminary studies indicate that the synthetic compound is highly unstable and reactive, the aldehyde was examined as a possible product of oxidation of DDOH by crystalline liver alcohol dehydrogenase (E.C.1.1.1.1).

The oxidation of DDOH was detected in a double-beam spectrophotometer by following the reduction of nicotinamide-adenine dinucleotide (NAD) at 340 nm in the presence of crystalline horse liver alcohol dehydrogenase (*3*). Because DDOH is insoluble in aqueous media, the compound was dissolved in 50 percent glycerolformal before its addition to the buffered incubation media. The resulting cloudy suspension prevented accurate spectrophotometric determination of reaction rates, but definite increases in absorbance were observed. No reaction was detected in the absence of enzyme or NAD. The reverse reaction, the reduction of DDCHO, was similarly observed with the substitution of reduced NAD for the oxidized form and by following the decrease in absorbance at 340 nm. Glycerolformal in the absence of DDOH also catalyzes the reduction of NAD, but the reverse reaction was not observed for glycerolformal.

Direct chemical evidence for the enzymatic oxidation of DDOH to DDCHO was obtained by formation of the *p*-nitrophenylhydrazone derivative. An incubation mixture was prepared containing 0.016*M* sodium pyrophosphate, *p*H 8.8, 0.008*M* NAD, and 2 mg of DDOH in a final volume of 6 ml. Crystalline liver alcohol dehydrogenase (2 mg) was added, and the mixture was incubated at 37°C for 30 minutes; then 0.01*M* *p*-nitrophenylhydrazine (0.5 ml) was added, and the mixture was shaken. A chloroform extract of the mixture was prepared and evaporated to a minimum volume. Portions of the extract were chromatographed on silica-gel plates with a mixture of benzene and petroleum ether (75:25) or benzene and ethyl acetate (95:5). The extract yielded a spot on the chromatograms whose R_F values, 0.28 and 0.12 for the respective solvent systems, corresponded to those of the authentic *p*-nitrophenylhydrazone derivative of DDCHO.

The identity of the derivative obtained from the enzymatic incubation was established by low-resolution, electron-impact mass spectrometry at 20 ev. High resolution measurements (*4*) confirmed the elemental composition of the ion fragments observed in the low resolution spectrum. An authentic sample of the *p*-nitrophenylhydrazone of DDCHO shows a prominent molecular ion at *m/e* (mass to charge) 399 and the base peak at 261. Other prominent ions of interest were *m/e* 249, 235, 226, 199, 200, 125, 122, and 111. The mass spectrum of the chloroform-extractable derivative was the same as that of the authentic sample of the DDCHO derivative. These findings strongly support the probability that DDCHO is a metabolite of DDT.

small

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Rate of Skin Absorption of Parathion and Paraoxon

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FREDRIKSSON[1] measured the rate of dermal absorption of paraoxon, a metabolite of parathion, in the cat by comparing the cholinesterase activity after dermal application of paraoxon with the activity after intravenous infusion of the compound. Since to our knowledge a similar experiment has never been done with parathion itself, this experiment was designed to measure its dermal absorption. A second objective was to compare the relative toxicities of parathion and paraoxon by the dermal and intravenous route in the same species.

Although the cat was used by Fredriksson,[1] the rabbit was chosen for these experiments because, like the cat, it is of a convenient size which allowed the cannulations to be made and blood samples to be taken; but, unlike the cat, the rabbit is readily available as uniform stock and is generally easier to handle.

Materials and Methods

Thirty male albino rabbits weighing 3.0 to 3.6 kg were carefully clipped on the right thigh or side area between the thigh and ribs. Because of the large area needed for the dermal application of parathion, the thigh was not large enough, and the side of the animal had to be used instead. The rabbits were clipped 24 to 48 hours prior to application of the compounds to allow at least partial reconstitution of the dermal barrier.[2] Animals having visible abrasions in the area of application were not used. The rabbits were kept under light pentobarbital anesthesia during an entire experiment. Each experiment lasted until the animal was showing severe symptoms of poisoning, usually about five to eight hours for parathion and three to six hours for paraoxon. Anesthesia was initially established with 65 mg/kg of pentobarbital sodium; subsequent injections were given as needed to maintain anesthesia. The carotid artery of each animal was cannulated for collection of serial blood samples. One thousand units of heparin were injected through the polyethylene cannula into the carotid artery as an anticoagulant. For intravenous infusion, the left femoral vein was cannulated.

Technical parathion (American Cyanamid) and technical paraoxon (Mallinckrodt) were used undiluted for the dermal application. The materials were tested for impurities by paper chromatography. The parathion was 95.7% pure, the remainder being: paraoxon, 0.5%; paranitrophenol, 0.3%; and three unidentified compounds totaling 3.5%. The paraoxon was 99.3% pure, the remainder being: paranitrophenol, 0.4% and an unidentified compound, 0.3%. Preliminary studies showed that the dermal application of undiluted technical parathion over a 144-sq cm area provided a suitable rate of plasma cholinesterase inhibition. The area was delineated with a collodion barrier applied to the side by using a plastic template as a guide. Undiluted technical paraoxon was applied to an 8.2-sq cm area delineated with two stainless steel rings fixed to the thigh area with collodion. An excess of parathion or paraoxon was applied within the outlined area with a syringe and spread with a polished glass rod. Because of variation in the characteristic of hair growth and the large area to be covered, the amount of parathion had to be varied between 1.25 ml and 1.75 ml per animal to produce a clear excess of the poison. Because there was less hair where the paraoxon was applied, the amount of it could be kept constant at 0.10 ml. Both compounds were applied just until runoff to ensure that an excess still remained at the end of the trial period. Fredriksson[3] showed with guinea pigs that, when an excess of poison is applied to a skin area, its toxicity is related to the area and not the amount of poison.

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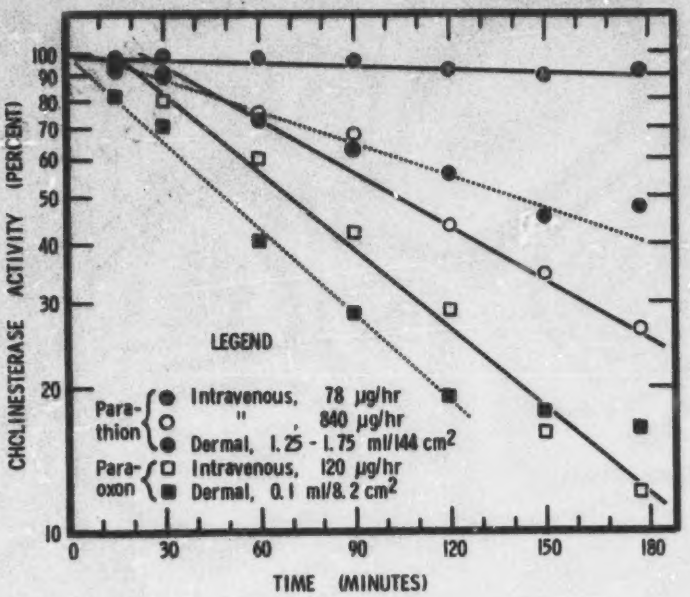

Inhibition of plasma cholinesterase activity in rabbits after application of parathion and paraoxon as shown in the legend. Time is in minutes after the beginning of administration.

For intravenous infusion, the technical parathion was dissolved in a small amount of propylene glycol and ethanol (1:1 v/v); technical paraoxon was dissolved in propylene glycol alone. Each solution containing the appropriate amount of toxicant was then diluted with an infusible fat emulsion (Lipomul) and shaken vigorously to form a stable emulsion. Under these conditions, the concentration of insecticide in the preparation remained constant for at least 24 hours. The formulations were infused through the polyethylene cannula into the femoral vein with an infusion pump at the rate of 1.2 ml/hour.

Although the vapor pressures of parathion and paraoxon are relatively low, respiratory exposure to them was prevented by placing the rabbits receiving dermal applications in a fume hood. Because of its high inherent toxicity, an additional precaution was taken with paraoxon by covering the rings with thin glass plates sealed with silicone grease.

Blood samples for cholinesterase determinations were taken before exposure, at 15 and 30 minutes after the beginning of exposure, and at half-hour intervals thereafter until completion of the experiment. Plasma cholinesterase was determined on the serial blood samples by the Michel method[4] which was modified by using 0.10 ml of 0.165 M acetylcholine perchlorate as substrate, 1.0 ml water, 0.04 ml of plasma, and 1.0 ml of a fourfold dilution of Michel's plasma buffer. Other conditions remained the same. Corrections were made for spontaneous hydrolysis of substrate in the absence of enzyme. In our hands, kinetic studies of this modification supported its validity for rabbit plasma at the level of activity observed in these studies.

Using the same procedure as that of Fredriksson,[1] linear transformation of the conventional logarithmic decay constant was used to calculate the rate constant for cholinesterase inhibition.

$$K = \frac{\log A_0 - \log A_1}{0.4343\ (t_1 - t_0)}$$

Where K is the rate constant, A_0 is the initial activity (at time t_0), and A_1 is the determined value at time t_1. The dermal absorption value was then determined according to the following formula:

$$\mu g\ \text{absorbed/minute/sq cm} = \frac{K_1}{K_2} \cdot \frac{B}{60} \cdot \frac{1}{C}$$

Where K_1 is the rate constant for the cholinesterase inhibition due to dermal absorption, K_2 is the constant for intravenous infusion, B is the rate of infusion of the compound in μg/hour, and C is the number of square centimeters of skin area over which the compound was applied.

Results

The logarithmic representation of the average cholinesterase inhibition curves for each treatment expressed as percentages of zero-time values appears in the Figure. Although the experiments were carried out for as long as eight hours, the part of each curve that approached 0% of normal is omitted. The data, including the inhibition rate constants and calculated dermal absorption rates, are given in the Table. The rate of cholinesterase inhibition was quite slow when the rate of infusion of parathion was 78μg/hour but increased to a very rapid rate when infusion was at the rate of 840μg/hour. It was found by comparing the rate

Dermal Absorption Rate of Parathion and Paraoxon as Measured by Cholinesterase Inhibition

			Inhibition Constant (K/minute)		Dermal Absorption Rate (µg/minute/Sq cm)	
Compound	Route	Dosage	Average	Range	Average	Range
Parathion	I.V.	78 µg/hour	0.00066(3)*	0.00046-0.00110		
	I.V.	840 µg/hour	0.0086(5)	0.0064-0.0120		
	Dermal	1.25-1.75 ml per 144 sq cm	0.0052(11)	0.0018-0.0350	0.059	0.021-0.39
Paraoxon	I.V.	120 µg/hour	0.0123(5)	0.0068-0.0130		
	Dermal	0.1 ml per 8.2 sq cm	0.0161(6)	0.0026-0.0[illegible]	0.32	0.052-1.23

* Number of animals shown in parentheses.

of inhibition from dermal exposure with the rate from intravenous infusion of 840 mg/hour that the dermal absorption rate of parathion was 0.021 to 0.39 µg/minute/sq cm with an average of 0.059 µg/minute/sq cm.

In order to make the determination of the dermal absorption rate more accurate by this technique, conditions were chosen such that the cholinesterase inhibition rate constants for the dermal and intravenous exposures were similar. Thus, the average K (inhibition constant) of 0.0086 per minute for intravenous infusion of 840 µg/hour of parathion and 0.0052 per minute for the dermal application of parathion on 144 sq cm of skin area compared quite closely.

The absorption of paraoxon was determined in a way similar to that described for parathion. The average rate constant for the infusion of 120 µg/hour of paraoxon was 0.0123 per minute and for dermal exposure on 8.2 sq cm of skin surface was 0.0161 per minute. From these rates the dermal absorption rate for paraoxon was found to be 0.052 to 1.23 µg/minute/sq cm with an average of 0.32 µg/minute/sq cm.

Comment

All rabbits from which data were used showed easily recognized signs of cholinesterase inhibition regardless of the route of exposure. The most frequent signs were muscle fasciculation (particularly at the site of dermal application), diarrhea, salivation, lacrimation, miosis, and respiratory distress. Three animals died during the experiment—one after the dermal application of parathion and two after dermal application of paraoxon. Whether the poison or the anesthetic caused these deaths could not be determined because symptoms of poisoning are at times difficult to recognize in anesthetized, restrained animals and because a few animals die under anesthesia without any exposure to a pesticide. The three animals were omitted from the data, although there was nothing strikingly different in their cholinesterase inhibition rates and although Fredriksson et al[5] observed no difference in the rate of cholinesterase depression between anesthetized or unanesthetized dogs given sarin.

The evidence supports the hypothesis that parathion reaches the rabbit's blood stream largely unaltered. Rabbit skin did not have the ability to convert parathion to paraoxon nor was the skin[6] or serum[7] of the rabbit able to hydrolyze the ester linkages of parathion and thus detoxify the poison. The cholinesterase inhibition observed in the present study represented inhibition caused by the direct inhibitor—presumably paraoxon—formed from the conversion of parathion which has been shown to occur mainly in the liver.[8,9] However, of the parathion that reaches the blood stream of the rat, only about one-sixth of the compound is converted to paraoxon; the remainder is detoxified and excreted as some form of the thiophosphate.[10] The rate of metabolism of parathion to paraoxon or other metabolites by the blood or certain organs should be similar (at equal blood concentrations of the compound) regardless of its route of entry into the general circulation. Assuming this to be true, the infusion of parathion into a peripheral vein can be used to approximate its rate of absorption from the skin. In the case of paraoxon, however, the measurement of dermal absorption in the rabbit by this method may not be as accurate because of the relatively rapid hydrolysis of the compound which can occur in the skin[6] as well as in the serum[11] or plasma[6] of the rabbit.

Comparison of the inhibition constants per square centimeter of skin area indicates that parathion was 55 times less toxic than paraoxon dermally ($2.0 \times 10^{-3}/3.6 \times 10^{-5} = 55$). Comparison of the constants per microgram of compound infused indicates parathion was ten times less toxic than paraoxon intravenously ($1 \times 10^{-4}/1 \times 10^{-5} = 10$). The marked difference in the penetration of parathion and paraoxon through the skin may be explained in part by known differences in their solubility. Malkinson[2] stated that the dermal absorption of most compounds is related to their water and lipid solubilities; absorption occurs most rapidly for those compounds which have a high solubility in lipid and also an appreciable solubility in water. Both parathion and paraoxon have high lipid solubilities, but the increased water solubility of paraoxon which is 100 times greater than that of parathion[12] may enhance its penetration into the animal.

The rate of dermal absorption as measured by the rate of cholinesterase inhibition varies greatly for both parathion and paraoxon. The range between the slowest and fastest observed rate of inhibition for dermal application at the same dosage was about twentyfold for each compound, while the variation for intravenous infusion of the same dosage was only twofold. A similar large variation was seen with dermal absorption of sarin by rabbits.[13] The marked variation of inhibition rates associated with dermal application was presumably due to marked differences in the function of the skin of individual rabbits with regard to absorption although it is difficult to know what part unrecognized injuries may play in the variation. The use of undiluted compounds eliminated the possibility of chemical disruption of the dermal barrier by solvents. Mechanical disruption was minimized by using care in clipping the skin and by a delay of 24 to 48 hours between clipping and application of the poison. If the degree of variation in permeability of human skin is similar to that of rabbit skin, the variability would help to reconcile (1) the sporatic occurrence of poisoning in people attributed by the authors to dermal exposure[14-21] and (2) the lack of effect from heavy dermal exposure of volunteers.[22]

The dermal absorption rate of paraoxon in the rabbit (0.05 to 1.23 μg/minute/sq cm) was in reasonably good agreement with that observed in cats (0.13 to 0.26 μg/minute/sq cm).[1]

In the studies on cats, Fredriksson[1] recognized a latent period of 10 to 15 minutes from the beginning of intravenous infusion to onset of cholinesterase inhibition and a latent period of 20 to 28 minutes following the start of dermal exposure. The first period was attributed mainly to the time necessary to reach a blood level of inhibitor great enough to produce detectable inactivation. The difference between the latent period following percutaneous absorption and the one following intravenous infusion was attributed to the time necessary for diffusion of the compound from the skin surface to the blood stream. Logically, such periods of latency should exist, but they could not be seen with the rabbits probably because of the marked variation in rates of inhibition between individuals.

Summary

The percutaneous absorption rates of parathion and paraoxon have been estimated in rabbits by comparison of the rate of plasma cholinesterase inhibition observed when the compound was infused intravenously with the rate observed when the compound was applied dermally. The average rate of dermal absorption was estimated to be 0.059 μg/minute/sq cm of skin area for parathion and 0.32 μg/minute/sq cm for paraoxon. Parathion was found to be roughly ten times less toxic intravenously and 55 times less toxic dermally than paraoxon. The difference in ease with which the two compounds penetrated the skin may be explained in part by known differences in their solubility. There was approximately a tenfold individual variation in permeability of the skin by both compounds since each produced only about a twofold variation in cholinesterase inhibition when administered at a constant dosage intravenously but about

a twentyfold variation when applied dermally at a constant rate.

An infusible fat emulsion under the trademark of Lipomul was supplied by Upjohn Co., Kalamazoo, Mich.

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Route of Absorption of DDVP after Oral Administration to Rats

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In a recent study, Gaines (1966) has demonstrated that the toxicity of the insecticide DDVP (*O,O*-dimethyl-2,2-dichlorovinyl phosphate) to rats is markedly lowered when it is infused into the hepatic portal venous system as compared with infusion into the systemic venous circulation. This finding allows us to postulate that there exists in rats a detoxification process for DDVP in the liver. In order to clarify the toxicity of DDVP, it then becomes important to know by what route the compound is absorbed if taken orally, i.e., whether it is absorbed primarily into the hepatic portal venous system or into the chyle and thence into the systemic venous circulation. If an oral dose of DDVP were absorbed into the portal venous system, then the animal would be afforded the protection of the detoxification system demonstrated to be present in the liver.

METHODS

Ten 350-400-g male Sherman-strain white rats were used in the experiment. Each rat was fasted at least 12 hours and was given 1 ml of condensed milk by stomach tube 30 minutes prior to anesthesia in order to facilitate thoracic duct cannulation. The rats were anesthetized with sodium pentobarbital, 5 mg/kg intravenously, and a polyethylene stomach tube was placed and sutured at the mouth with presence of the tip in the stomach confirmed by palpation. One or more of the following procedures was performed on each rat:

A. Cannulation of the portal vein through a distal intestinal vein with a PE 60 catheter, the tip lying in the *porta hepatis*.

B. Cannulation of the thoracic duct with a PE 10 catheter with the tip in the cisterna chyli (Bollman *et al.*, 1948).

C. Cannulation of the jugular or tail vein with a PE 60 catheter, or, in two cases, bleeding without cannulation from a severed tail tip.

The rats were placed prone in individual restraining cages (Bollman, 1948).

Each rat was infused through its stomach tube with P^{32}-labeled DDVP.[1] The DDVP was formulated in a vegetable oil emulsion[2] at a concentration of 18 mg/ml and infused with a pump[3] at a rate of 0.068 ml/minute, a schedule which in most rats causes cholinergic symptoms in 15 minutes and, if continued, death in 30 minutes. The infusion was stopped for each rat when generalized fasciculations appeared.

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[1] Obtained from The Radiochemical Centre, Amersham, England.

[2] Lipomul®, Upjohn Company, Kalamazoo, Michigan.

[3] Harvard Apparatus Company, Dover, Massachusetts.

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Samples of blood or chyle were taken at standard time intervals, varying from 2 to 5 minutes, depending on the rate of flow of blood or chyle of each rat. Each sample was at least 0.1 ml, and a control sample was taken in each case before infusion was begun. Samples were collected in dry heparinized test tubes.

The activity of the radioactive DDVP was 0.0076 mc/mg at the time of standardization, and all experiments were performed on the same and the following 4 days. Standard curves[4] were obtained for radioactive DDVP in 1.0 ml of water to which was added 0.1 ml of blood or 0.1 ml of chyle. Self-absorption by the blood and chyle was found to be minimal. Each sample was analyzed by adding a 0.1 ml aliquot to 1.0 ml of distilled water, shaking, and reading counts per minute in the dip counter after a background reading with a dry dip counter had been obtained.

All animals were autopsied to confirm the placement of catheters.

RESULTS

It is felt that the results are most meaningful when the mean of the various times of onset of cholinergic symptoms and of death is considered as the standard time for all cases. Table 1 gives the levels of DDVP obtained at quarters of the time from start of infusion to onset of symptoms (16 ± 1.5 minutes) and from onset of symptoms to death (12.0 ± 2.0 minutes). When the mean values for each experimental group are plotted graphically (Fig. 1), it is evident that the vast majority of the DDVP detected is in the portal venous blood, that the amount in the systemic blood is very little in comparison, and that the amount in the chyle is even less.

DISCUSSION

DDVP is seen to appear promptly, accumulate at a rapid rate, and reach relatively high levels in the portal blood. None of these statements is true concerning either the systemic blood or the chyle, both of which show a slow rate of accumulation and maintain relatively low levels. The curves for systemic blood and chyle are very nearly parallel, with the values for chyle slightly lower. The presence of DDVP in the systemic blood and later in the chyle can be accounted for by the amount of the compound reaching the general circulation after passage through the liver. The variation in values within a group is believed to be accounted for by individual variations, differences in exact site of placement of the several catheters, and errors inherent in the radioassay method employed. The dramatic difference between the levels of DDVP in the portal blood and those in the systemic blood and chyle confirms the hypothesis that DDVP taken orally is absorbed primarily, if not exclusively, by the hepatic portal venous system.

Support for the above hypothesis was also provided by some preliminary studies and observations. Prolongation of time to onset of symptoms and to death was noted where animals were infused with the portal vein tied off, and such prolongation was also noted when a PE 60 catheter in the portal vein was allowed to drain off most of the portal blood.

In the course of the present experiment, two postmortem samples of portal blood with a high concentration of radioactive DDVP were collected in nonheparinized tubes, and differential determinations were made on the serum and red blood cell fractions.

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[4] The instruments used were a model 1620B analytical count ratemeter and model 121 dip counter detector, Nuclear-Chicago Corporation, Des Plaines, Illinois.

TABLE 1

Differential Absorption of Orally Infused Radioactive DDVP

Sample	Rat no.	Micrograms of DDVP per 0.1 ml of sample								Actual	
		1/4 O–S[a]	1/2 O–S	3/4 O–S	S	1/4 S–D[a]	1/2 S–D	3/4 S–D	D	Time to S[b]	Time to D[b]
Portal vein 1	1	0.83	0.94	2.16	4.24	6.83	7.06	7.17	7.51	20	41
Portal vein 2	2	1.01	0.96	1.06	1.77	4.13	4.67	5.21	5.74	15	28
Portal vein 3	5	0.45	1.78	2.45	3.78	4.40	4.22	3.10	3.08	12	20
Portal vein 4	7	0.13	1.99	5.30	5.55	6.55	12.0	16.0	20.0	21	38
Portal vein 5	10	0.05	0.14	0.75	5.23	6.57	7.91	9.14	9.25	20	21
Portal blood Mean ± SE		0.50 ± 0.19	1.15 ± 0.33	2.35 ± 0.80	4.10 ± 0.67	5.70 ± 0.60	7.20 ± 1.4	8.10 ± 2.2	9.10 ± 2.9		
Tail vein 1	3	0.05	0.05	0.05	0.05	0.07	0.14	0.17	—	23	44
Tail vein 2	5	0	0.22	0.43	0.52	0.60	0.97	1.27	1.30	12	20
Tail vein 3	6	0	0	0.10	0.30	0.40	0.50	1.75	—	15	30
Jugular vein 1	9	0.05	0.10	0.17	0.15	0.22	0.30	0.34	0.38	7	11
Systemic blood Mean ± SE		0.05	0.10 ± 0.05	0.20 ± 0.09	0.25 ± [illegible]	0.30 ± 0.11	0.50 ± 0.18	0.85 ± 0.38	0.85		
Chyle 1	4	0	0.05	0.11	[illegible]	0.46	0.67	0.88	1.10	18	35
Chyle 2	6	0	0.13	0.05	[illegible]	0.05	0.14	0.19	0.33	15	30
Chyle 3	8	0.05	0.05	0.05	[illegible]	0.20	0.42	0.48	0.53	14	17
Chyle Mean ± SE		0.05	0.05 ± 0.04	0.05 ± 0.03	0.10 ± 0.07	0.20 ± 0.13	0.40 ± 0.15	0.52 ± 0.20	0.65 ± 0.23		
Total time Means ± SE										16.5 ± 1.5	28.5 ± 3.5

[a] O = onset of infusion; S = appearance of first symptoms; D = death.

[b] Minutes.

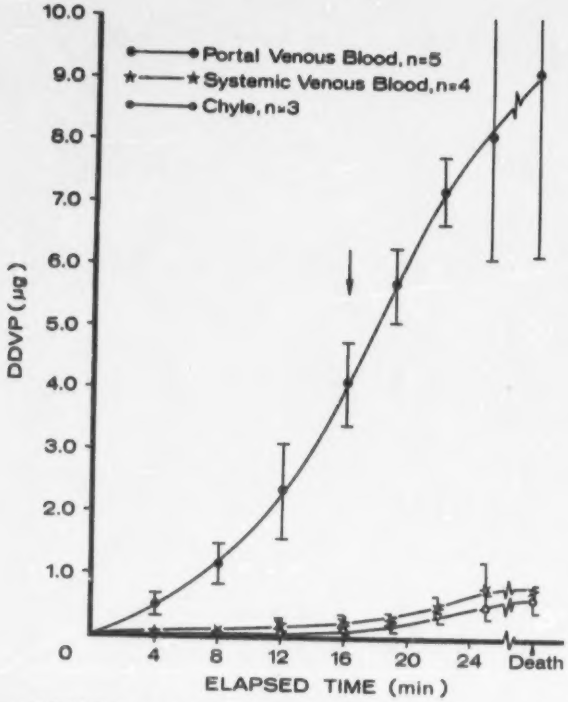

FIG. 1. Amount of DDVP in 0.1 ml samples of portal blood, systemic venous blood, and chyle of rats at average intervals after the beginning of oral infusion with the radioactive compound. Each point is the mean for the indicated number of values, and the vertical line through it indicates the standard error. The arrow indicates the time at which symptoms began.

These were found to be almost identical in both cases, indicating an equal partitioning of DDVP between serum and red blood cells.

SUMMARY

Ten rats were infused by stomach tube with radioactive DDVP while timed samples of portal blood, systemic venous blood, and chyle were taken. Levels of DDVP were determined in all samples and were found to be much higher in portal blood than in either systemic blood or chyle. They were slightly higher in systemic blood than in chyle.

It is felt that these findings support the hypothesis that, in the rat, DDVP taken orally is absorbed primarily if not exclusively by the hepatic portal venous system, and, therefore, is routed through the detoxification processes of the liver before reaching the systemic circulation.

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Epidemiology of Poisoning by Chemicals

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During the pre-World War II era, the hazards associated with the use of many common chemicals went unheeded by the nations of the world. In 1940, a British article was published implicating aspirin in 43 suicides, 8 accidental deaths, and 14 unclassified fatalities in Great Britain during that year alone.[1]

A series of accidental deaths from acetylsalicylic acid were reported from the United States in 1950.[2] At that time investigations revealed that from 1926-1943 a total of 227 persons had died from the consumption of excessive amounts of this chemical. These deaths were associated with the increase in production of just one of the salicylates—acetylsalicylic acid—which had exceeded 9 million pounds in one year.

Medicants of various kinds have been shown to play a significant role in childhood accidents and deaths. For example, oil of wintergreen was responsible for 6 deaths among children in England from 1931-1939, and 17 from 1940-1949.[3] The same chemical accounted for 28 deaths among children in the United States in 1951, and 34 in 1952.[4] Other products of concern have been broadly classified into categories such as disinfectants, antiseptics, household poisons, poisonous plants, and "miscellaneous."

A review of the accidental poisoning problem in the United States,[5] prompted by the findings of British investigator Douglas Swinscow,[3] revealed a total of 3,659 fatalities among children during the period 1940-1950. This represented a mortality rate four times greater than that among children in England during the same period, which was attributed, initially, to racial differences between the two populations. Medicants (drugs) accounted for approximately two-thirds of the cases in England, but about one-third of the cases in the United States. In the United States during the period 1949-1950, aspirin and the salicylates caused a total of 113 deaths in children under 5 years of age; barbiturates ranked second and accounted for 31 deaths. Iron poisoning, common in England, is rare in this country, and only two cases were found in this review. In one year, more than 100 deaths in the less than 5-year-old group were due to ingestion of petroleum products, one of the major factors responsible for increasing the rate in the United States over that in England.

In 1953, a Scottish researcher reported on a detailed investigation of poisoning episodes in that country[6] which showed that the number of accidental poisonings seen at the hospitals in Edinburgh had risen from 5 cases in 1931 to 50 cases annually by 1951. This stimulated an investigation into the accidental poisonings seen at the hospitals in Edinburgh and Aberdeen, and the author reviewed 502 cases that had occurred during that period. The principal agents responsible

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for illness among children in that study population are shown below:

Agent	*No. of Cases*
Disinfectants	39
Kerosene	31
Turpentine	25
Bleach	22
Barbiturates	36
Iron Preparations	24
Salicylates	20
Camphor	29
Laburnum	15

Concern about the problem of accidental poisonings in the United States prompted creation of Poison Control Centers.[7, 8] The first center was established in Chicago in the latter part of 1953 and operated on a city-wide basis. This center combined the efforts of the Departments of Pediatrics of all five medical colleges in Chicago, the Chicago Board of Health, the State Toxicological Laboratories, the Division of Services for Children of the University of Illinois, and the six major teaching hospitals in Chicago. An advisory committee that included representatives of the American Medical Association, the National Safety Council, and the Food and Drug Administration was formed. Each participating hospital was provided with a summary of references on the basic toxic constituents of the thousands of materials used in average households. Current knowledge on treatment for ingestion of these materials was also made available to each of the participating hospitals. A pediatrician was designated as Poison Control Officer, responsible for liaison between the central committee and participating groups, assuring the availability of necessary equipment and supplies in emergency rooms, orienting staff members to the problems of poisoning, and reporting to the Chicago Board of Health.

By March, 1958, 124 centers were in operation in 40 states,[7] and this was increased to 580 centers in 49 states in 1960.[9] The general purpose of the centers is to minimize the danger of toxic substances by improving and expanding efforts for the prevention and treatment of poisoning.

In 1957, the Public Health Service reacted to the chemical poisoning problem by establishing the National Clearinghouse for Poison Control Centers.[8] This group maintains detailed lists of the ingredients of all household products and drugs and provides the information to the local centers. Conversely, the local centers provide reports to the Clearinghouse, on any new poison hazards they discover for dissemination to all other centers. The Clearinghouse conducts research activities, investigates and analyzes poison cases, and provides information on these and other activities to the local centers through a monthly newsletter. The Poison Control Centers provide the framework for detailed studies of the chemical poisonings in many communities throughout the United States.

Scientific articles published since the Poison Control Center network began include several dealing with epidemiologic analysis of case reports submitted to the National Clearinghouse[10, 11] and others reporting specifically on the chemical poisoning problem in selected metropolitan areas such as Chicago[12], New York[13-15], Salt Lake City[16], and Syracuse[17, 18]. One of these reports[10] was an analysis of 3,926 cases of accidental poisoning. The age group of 5 and under accounted for 96 percent of the cases occurring in children under age 15 and 86 percent of the total. Males were reported to be involved in 53 percent of the total cases. Medicinal agents (aspirin, barbiturates, non-barbiturate sedatives, tranquilizers) were the offenders in 2,152 cases (54.8 percent). Household preparations such as soaps, detergents, and bleach accounted for 561 ingestions (14.3 percent), followed by pesticides, 315 cases (8 percent); the remainder (22.9 percent) were attributed to a variety of other products.

A paper by Howard M. Cann and colleagues reviewed in detail the results of 15,094 individual case reports submitted to the Clearinghouse.[11] A similar pattern of age distribution was shown, with approximately 90 percent of the cases occurring in children less than 5 years of age. Internal medicants were ingested in 41 percent of the accidents, and aspirin was reported in over 50 percent of these cases. Where the type of aspirin was known, the "baby" type accounted for 84 percent of this total. Household cleaning and polishing agents were ingested in 17 percent of all the accidents. Ten percent of all the reported ingestions involved pesticides—the majority of which were insecticides. This article differed from those previously published in that an attempt was made to determine the type of container in which the ingested substances were found at the time of the accident. In one-third of the total cases, the chemicals were not found in the original container. Forty percent of the poisoning accidents occurred in the kitchen, 22 percent in the bedroom, and 12 percent in the bathroom; the remaining incidents occurred in the basement, living room, garage, and other areas.

The report on the study in Chicago[12] showed the results of analysis of 1,033 cases of poisoning reported from 19 hospitals between December, 1953, and May, 1955. A pattern characteristic of that shown earlier was found. Medicines were the primary agent, accounting for 523 (50.6 percent) of the incidents; of these, 210 were attributed to aspirin, and 196 cases involved cleaning, polishing, and sanitizing agents, with bleach the most frequently ingested chemical in this category. Poisoning with pesticides occurred in 107 (10.4 percent) of the total cases; insecticides were the primary agent in this classification, followed by rodenticides. There were six accidental deaths in children under 10 years of age, and six fatalities in persons 22-48 years of age which could have been either accidental or suicidal. In the majority of poisoning cases, the offending agents had been stored in the kitchen; in the remaining cases, they had been kept in the bedroom, bathroom, and other locations, in that order of priority. A predominance of the cases occurred among persons residing in low socio-economic areas; however, the author admitted that the possible bias in any conclusion drawn from this fact would be appreciable.

Chemical poisonings, excluding those due to gas, are the third leading cause of death in children under 6 years of age in New York City.[13-15] In 1954, 454 cases of chemical poisoning occurred in children under 16 years of age in that city. Drugs were responsible for 47 percent of all poisonings, with aspirin as the principal agent and barbiturates as the second leading cause in this category. Bleaches were the primary agent in the household preparations class. Thirteen deaths were reported among children as the result of ingestion of lead-based paints; this finding corroborates similar investigations of lead-paint poisoning episodes in other areas of the United States such as Philadelphia[19], Baltimore[20], St. Louis[21], Cleveland[22], and Chicago[23].

A 1955 study confirmed the problem of poisonings in children in New York City.[14] Twenty-one attempted suicides with chemicals were reported in children ranging from 11 to 16 years of age. Eighteen were girls and three boys. Age 13 was a high-risk category, accounting for nine incidents. Aspirin was responsible for 10 poisonings, followed by barbiturates, iodine, bleach, tranquilizers, antiseptics, and other agents. While no fatalities were reported among this group, seven fatal cases were reported in children ranging in age from 10-24 months.

A substantial increase in the number of poisoning incidents in New York City between 1960 and 1961[15] was attributed to improved reporting by physicians. Sixty-six fatalities from poisonings occurred during this period, with barbiturates as the chief cause. Ten fatalities from barbiturates, mainly suicides, were reported in the adult group. Six fatalities occurred in children 5 years old, four from lead poisoning, one from talcum powder ingestion, and one from cough syrup. The reported number of cases rose from 5,070 for a six-month period in 1960 to 6,414 for the same time interval in 1961.

The study in Salt Lake City, Utah, in 1953 and 1954 revealed that 479 persons were treated for chemical poisoning in those years.[16] Of this number, 311 were children and 168 adults. Drugs were by far the most common cause of poisoning in both children and adults. Aspirin accounted for 27 percent of all the cases in children. Four persons died from ingestion of chemicals—one child from cyanide and three adults from cyanide, strychnine, and alcohol.

An attempt was made to study the prevalence and characteristics of accidental poisoning in an urban population in Syracuse, N. Y.[17, 18] The authors obtained their data by direct interviews with a 2 percent sample of the total population of this city. Thus, 1,069 families with 4,069 members were selected. This group was considered to be reasonably representative of the total city population in age, sex, and socioeconomic status.

A total of 107 poisoning episodes were recorded among the study population. Medicines accounted for 63 of the poisonings, 33 of which involved aspirin. The remaining ingestions (44) were due to household products, petroleum-based chemicals, pesticides, paint and paint products, cosmetics, and other agents. Physicians were consulted in only 79 of the total cases. Some of the persons interviewed were not aware of the hazards of aspirin, and only about 66 percent of these ingestions were brought to the attention of a physician. This contrasted with the experience in other types of poisonings where 80 percent or more had sought medical assistance. The authors were not able to demonstrate any geographic or socioeconomic aggregation of cases.

M. A. Heasman presented morbidity data from England and Wales for 1958 and mortality data for the period 1954-1958.[24] The 1958 morbidity data were obtained from a 10 percent sample of all patients discharged from the National Health Service hospitals (excluding mental hospitals). From these records, 1,000 consecutive cases of poisoning were extracted. A total of 218 of these cases occurred in children under 15 years of age, about 50 percent of which involved the ingestion of pills, tablets, and other medicinal substances. In this category, aspirin accounted for 44 of the cases; barbiturates, 28; and other substances, 33. Turpentine, paraffin, and disinfectants ranked highest among the household products group, with 18, 16, and 7 cases, respectively. Berries and plant substances were responsible for 8 cases. Although there were no deaths among these cases, fatalities in this age group in England and Wales during the period 1954-1958 averaged 44 per year. As with the hospital admissions, aspirin and salicylates were the most common causes of death. Iron preparations were responsible for 12 deaths, and 10 deaths were attributed to strychnine.

Attention has been focused on the role of pesticides as causative agents in poisonings.[25-28] The extent of injury from pesticides on a nationwide scale is difficult to determine, but California publishes a detailed tabulation of nonfatal injuries from economic poisons annually.[25] The difficulty in compiling reliable pesticide poisoning statistics has been due to the lack of uniformity in identifying and recording agents responsible for injury. For instance, from a total of 446 entries listed under the category of poisoning in the International Statistical Classification of Diseases, Injuries and Causes of Death, only 17 items relate to pesticides. There are, however, over 400 basic pesticide chemicals and approximately 45,000 formulations now available. In a study of pesticide mortality, it was shown that 196 deaths were reported in 1946, 154 (1949), 152 (1950), 132 (1952), 133 (1953), 105 (1954), and 140 (1955).[25]

Data submitted by various reporting groups such as industrial commissions and Poison Control Centers support the conclusion that only the more familiar materials used as pesticides are included in this category. A later report[26] included data on mortality from pesticides for the years 1956 to 1960. Pesticides were reported as the cause of death in 141 cases (1956), 111 cases (1958), 130 cases (1959) and 103 (1960). A paper published in 1964[27] speculated that the rate of accidental deaths from pesticides was about one per million persons each year. It was further indicated that there had been no significant change in the death rate associated with the increased use of the new pesticides.

Results of detailed investigations conducted in certain high pesticide-use areas of the United States, however, showed a higher death rate. For example, in Dade County, Florida, which has a population of 1,040,000, 89 deaths due to pesticide ingestions were reported from 1956 to

Table 1
Reported Poisonings by Pesticides — United States* 1968 - 1970

	Cases			Deaths		
Pesticide	*1968*	*1969*	*1970*	*1968*	*1969*	*1970*
Insecticides	2,919	2,929	2,913	11	14	13
Rodenticides	1,185	1,192	1,132	4	6	6
Fungicides	99	90	106	0	0	0
Herbicides	254	281	287	9	6	1
Mothballs	922	813	933	0	0	1
Miscellaneous	360	397	358	0	0	0
TOTAL	5,739	5,702	5,729	24	26	21

*Source: Poison Control Center Data

1964. This statistic included 23 cases designated as accidental, 52 suicides, and 4 homicides. Parathion, an insecticide not specifically listed in earlier papers, was shown to be responsible for 9 of 10 deaths among children over a four-year period.[28] A unique problem in Dade County is the illegal door-to-door sale of parathion in unmarked containers. This activity in itself has been shown to be responsible for the deaths of several children. Data on total poisonings from pesticides reported in three recent years are shown in Table 1.

The etiology of accidental poisoning episodes has been the subject of considerable investigation. In addition to the more common safety hazards from improper storage of chemicals, other facets of the problem have been explored; for example, the role of oral abnormalities. J. O. Craig[29] showed that children who poison themselves can be divided into two groups, those who take the poison (usually in the form of attractive tablets or liquid), but who only taste or chew the substances and, in the majority of cases, reject them before harm results; and those who show exaggerated oral traits, amounting to pica in about 50 percent of the cases, and poison themselves with tablets of any kind. Tablets attract these children more than liquid and candy-flavored tablets such as aspirin or ferrous sulphate may be ingested in lethal quantities. The risk of death to children in this group is greater than in the first group.

An attempt was made by Australian workers in 1956 to correlate social factors with accidental poisonings among children.[30] Results of their study indicated that negligence or carelessness by parents was an uncommon cause of poisoning. In follow-up investigations, the authors found that 70 percent of the poisoned children came from homes whose parents were conscientious in caring for their children; 63 percent of the 155 cases were from "standard" homes, and 68 percent of the fathers were professional people, businessmen or skilled tradesmen. The smaller number of children came from poorer families, belonging to the laboring or semi-skilled groups who had a lower material standard of living.

In other attempts to establish possible social factors in accidental poisonings, investigations were made of repetitive poisoning cases. One of these investigations[31] was based on the study of a group of 100 children who had experienced one episode of accidental poisoning. A group of matched controls were selected from a previous randomized population derived from the city of Syracuse.[18] The poisoned group was found to experience many more subsequent episodes of poisoning than the control group. These children were more active and mischievous, were more likely to experience falls, and were more frequently separated from their parents. The girls in the repeater group were described as being more boyish, and both sexes walked earlier than members of the control group.

In a later study of the families of 20 poison repeaters, 19 single ingesters, and 13 controls, no correlation was apparent between repeated episodes of poisoning in children, and religious affiliation, income, geographic setting, or family size.[32] Contrary to the study reported by Wehrle *et al.*[31], these investigations showed no relationship between repetitive poisonings and accident proneness, pica, environmental hazards, or lack of parental supervision. The ingestion of poisons seemed to be the result of purposeful behavior on the part of the child. Possible causes of the first ingestion were considered to be the result of the child's negativism, imitation of the parent's pill taking, or confusion with food. Subsequent ingestions were described as methods to get attention.

Baltimore and Meyer[33] suggested that improper storage and parental ignorance of toxicity were secondary environmental factors in accidental poisonings; although no statistical difference in poisoning incidents could be proved. A statistically significant relationship was found, however, between "pica" and "daredevilness" in children and poisoning accidents.

Scientific literature contains a variety of published reports on methods and programs for the prevention of accidental poisonings. The most successful program for reducing child deaths was the network of Poison Control Centers. Their influence on accidents is more difficult to determine since the Centers are not located in all urban and rural areas and are operated on a voluntary-reporting basis.

Other proposals for the prevention of poisoning incidents have related to labeling of containers for chemicals.[34, 35] The need for precautionary labeling of chemical products was first formally expressed by the American Medical Association in 1884 with the adoption of a resolution urging Congress and state legislatures to enact laws requiring containers of lye to bear a poison label.[35] Specific warning statements are required on various types of products under the Federal Food, Drug, and Cosmetic Act, the Caustic Poison Act, and the Federal Insecticide, Fungicide, and Rodenticide Act.[25] The influence of labeling in decreasing accidental poisoning among children is difficult to assess however.

A study, made to determine the degree of "attractiveness" of certain medicants, particularly aspirin, to children, indicated that bright colored tablets were the most popular, with magenta ranking first.[36] Brown placed high among the study population, presumably because of its similarity to chocolate. Wine- and black-colored tablets were low on the list, therefore, the researcher proposed that aspirin and other drugs be incorporated in black-colored tablets and that tablets be dispensed in plastic strips which are difficult for children to tear.

In a study reported in 1959[37], the use of safety closure caps on drug containers was proposed. Fourteen different types of safety caps were investigated over a period of three years, and two caps were finally selected for comparison with the conventional screw cap in a test area of 1,600 homes that had children under 5 years of age. Over 93 percent of the children in the survey had difficulty in removing the two experimental caps, and it was concluded that a safety cap was an important and practical safety measure. In a study in the Tacoma, Wash., area[38], the author found that, after the introduction of bottle closure devices, the rate of accidental poisonings from tablets dropped from one per 5,100 prescriptions to one per 62,300 prescriptions. Poisonings due to children's flavored aspirin were reduced by 97 percent. Similar results were reported from a Canadian investigation.

In a detailed study of the etiology of a group of 100 poisoning incidents, Jensen and Wilson[40] found three circumstances, singly or in combination, that were common to 86 of the cases. In 26 cases, a person other than the parents (siblings, other children, neighbors, relatives, friends) played an important part in the episode. These individuals either made it possible for the poisoned child to reach the substance or failed to properly store the chemical. In 31 cases the parents were not aware that the child could climb, open doors, unscrew caps, or perform other tasks necessary to obtain the agent. In 53 cases the poison was not in its usual location, either because it was in use, because persons other than the parents had left it out, or because the parents themselves were careless.

Studies have shown that many accidents could be avoided if parents would refrain from taking medicines in the presence of their children.[41] A detailed study in the Norwalk, Conn., area supported the findings that children will imitate their parents in this respect. It was shown that of the children poisoned by medication other than aspirin, 67 percent frequently observed their parents taking pills. This was found in only 13 percent of a comparison group who ingested "baby" aspirin. The investigator suggested that this factor in poisoning incidents could be minimized by proper parent education.[42] Application of the traditional approaches to public health education has been suggested to reduce accidents from chemicals.[43] Included among the educational techniques which have been employed are films, exhibits, lectures, demonstrations, and the establishment of poison prevention programs with community health councils, Parent-Teacher Associations, 4-H Clubs, and similar organizations.

A study conducted in Charleston, S. C., in 1962 and 1965[44] demonstrated the practicality of a concentrated, community-wide program to reduce the incidence and severity of accidental poisonings. This survey pointed out that lack of awareness by adults still exists at all socioeconomic levels. One of the newest approaches to poison prevention is Poison Central[45] established in Kentucky in 1962, which provides a master toxicology information and service facility for the state system of regional poison information and treatment centers. Poison Central operates on a 24-hour basis seven days a week and provides toxicologic services not available at regional centers or at local hospitals and clinics. It also provides readily available services for areas not served by Poison Control Centers.

The deliberate ingestion of chemical agents or the so-called "therapeutic misadventures" or self-poisoning episodes, with or without suicidal intent, is a problem of great concern. This problem, particularly among young individuals, was noted in New York City after the Poison Control Center was set up in 1955.[46] The author of a paper on this subject in the New York area indicated that 299 suicidal attempts by poisoning were reported for individuals under 20 years old. These episodes took place between March, 1955, and December, 1958. Females accounted for 232 cases or 78 percent of the total. Puerto Ricans, who compose 11.8 percent of New York City's population, accounted for 28 percent of the attempted suicides. The primary agents involved were aspirin (84 cases—28 percent), barbiturates (47 cases—16 percent) and tranquilizers (36 cases—12 percent); the remaining cases were attributed to bleach, iodine, lye, insecticides, and other chemicals. A definite seasonal pattern was noted, with the incidence lowest in autumn and highest in spring. The type of agents used in the New York City cases have been implicated in similar episodes elsewhere.[47]

James Toolan speculated on some of the predisposing causes to suicide.[48] In his report, he discussed two cases involving the ingestion of chemicals. One of these was a 14-year-old male who swallowed an unidentified toxic agent to prove that he was invulnerable. Another was a 12-year-old girl who attempted suicide by taking a large amount of sedatives, because she dreamed of her dead mother and wanted to join her.

The hazards to health from the availability of proprietary drugs like scopolamine have been a matter of concern because of the involvement of these chemicals in self-poisoning incidents. This chemical has been the subject of many reports, including one from Casper, Wyo.[49] where six cases involving suicidal ingestions were treated in a six-month period. The authors of that report take exception to advertisements that emphasize the "safe" or "harmless" character of sedatives containing this chemical. Other investigators[50] have held similar views concerning this agent. The toxic dose is reported to be about 3-5 mg. (12-20 tablets).

From the experience of these researchers, self-induced drug intoxications usually involve five main classes of agents—barbiturates, salicylates, glutethimide, meprobamate, and phenothiazines. Their experience also indicated that ethylene glycol, bromides, and scopolamine preparations are frequently ingested with suicidal intent.

A study dealing with the various aspects of self-poisoning in the Chicago-Cook County area showed that in 1964, 468 persons were treated for deliberate poisonings in the adult emergency department at Cook County Hospital.[51] The preponderance of persons in the group were females (333—71 percent). Persons less than 45 years of age made up 84 percent of the group; more than half of these were less than 25 years old and a large number were teenagers. The patients ingested 102 different drugs and chemicals. Barbiturates were again the agent of choice, followed by the salicylates and tranquilizers. Rubbing alcohol, bleach, antiseptics, and rodenticides were also used as self-destructive agents.

Two comprehensive studies on self-poisoning episodes have been published by the British in recent years.[52, 53] In the Oxford area, it was shown that 502 females and 200 males, ranging in age from 11 to 85 years, were admitted for treatment of self-induced poisonings between 1962 and 1965. Females outnumbered males in all age groups. The authors reported that there were 40 double admissions, seven triple, two quadruple, and one sextuple. The main poisons used were barbiturates (45 percent of the cases) and aspirin (26 percent). The use of barbiturates rose steadily with age from 23 percent in the age group under 20 to 60 percent in persons aged 50 and older. Over the same age span, the use of aspirin declined from 49 to 12 percent. The highest rate of self-poisonings was observed among married females under the age of 20. Unmarried girls in the same age bracket had a lower rate, which was statistically significant. Married women showed slightly higher rates than single women at all other ages, but both groups showed diminishing rates above the age of 25. Of the total number of persons in the study population, eight deaths were recorded.[52]

In the Cardiff, Wales, area, all cases of intentional poisoning admitted for treatment between the years 1950 and 1965 were studied.[53] During this period 1,736 deliberate poisoning episodes (classified as "serious attempt", "gesture", and "doubtful") were recorded, involving 1,541 patients. The number of female patients (1,048) was greater than the number of men patients (493) in all age groups, but the greatest number for both men and women was in the age group 25-45; these numbered 199 and 436, respectively. The next highest age group for women was 0-25 years and, for men, 45-65 years. Multiple admissions occurred in 14 percent of the serious attempts, 7 percent of the gestures, and 11 percent of the group classified as doubtful. Attempts were made to associate significant organic illnesses with the poisoning gesture. Twenty percent of the individuals did have some illness at the time of the episode. Twenty-six percent of the patients had been under psychiatric care, and 38 percent were known to have been suffering a mental illness of one kind or another, mainly depression before the episode. The agents used in these incidents were barbiturates, salicylates, and coal gas (carbon monoxide), which together accounted for 70 percent of the attempts. The number of fatalities that resulted from the ingestion of these and other agents was not specified.

The preference of barbiturates over other methods of committing suicide was pointed out in a report of an intercultural study of the suicide problem in Los Angeles, Calif., and Vienna, Austria. In one recent year, barbiturates and other drugs and chemicals were responsible for 30 deaths in Los Angeles and 31 in Vienna. The use of chemicals ranked after domestic gas and hanging in Vienna and shooting in Los Angeles.

The incidence of suicidal attempts (non-fatal) is difficult to establish. In a study by Parkin and Stengel[55] to determine the incidence of suicidal attempts in Sheffield, England, for the years 1960 and 1961, the following definition was adopted: "A suicidal attempt is an act of self-damage undertaken with the apparent intention of self-destruction, however half-hearted and ineffective." Results of their study showed that there were a total of 358 suicidal attempts admitted to hospitals in 1960 as compared to 39 actual suicides; in 1961, attempted suicides treated in hospitals totalled 359 as compared to 47 actual suicides. For 1960, 45 percent of the attempted suicides were attributed to narcotics, 15 percent to salicylates, and 20 percent to other chemical agents; for 1969, 40 percent were attributed to narcotics, 16 percent to salicylates, and 22 percent to other chemical agents. The remaining attempts for both years were due to hanging, slashing of the wrists, and other means.

The prevention of deliberate poisonings involves the application of numerous techniques such as proper counseling of persons with psychological problems and maintenance of proper controls over drug items. Persons ill from an overdose of a toxic chemical must receive proper diagnosis and treatment, and surveillance should be maintained over individuals in high-risk categories.

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Studies on the Toxicity of O,O-Dimethyl-2,2-Dichlorovinyl Phosphate (DDVP)

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The isolation, identification, and synthesis of O,O-dimethyl-2,2-dichlorovinyl phosphate (DDVP), an insecticidally active organic phosphorus compound, have recently been reported.[1] In view of the potential widespread application of the compound, studies have been carried out at this laboratory on the mammalian toxicity of DDVP.

Methods

The DDVP used was a technical grade material prepared by the Chemistry Section of this laboratory, except where material of another purity was specified.

Experimental animals used in this study were white rats of the Sherman strain, adult rhesus and cynomolgus monkeys of both sexes, and adult female Rhode Island Red hens weighing between 2200 and 2938 gm.

The single oral doses of DDVP were given in peanut oil solution by stomach tube. The dosage rate was kept constant at 0.005 ml/gm. of body weight. The dosage level was varied by changing the concentration of DDVP in the solution.

The dermal doses were applied in xylene solution to an area of clipped skin between the shoulder blades. The skin was washed with a 1:1 mixture of alcohol and acetone before the poison was applied. A constant dosage rate of 0.0016 ml/gm. of body weight was used. The L. D.$_{50}$ values were calculated by the method of Litchfield and Wilcoxon.[2]

A Peet-Grady chamber was used as the exposure cell in a part of the studies of the respiratory toxicity of DDVP. The walls and ceiling of the experimental chamber were sprayed with a xylene emulsion containing 2.5% DDVP and 6% sugar at the rate of about 100 mg. of toxicant per square foot. A second chamber was treated similarly with the same volume of a control formulation. Two very similar separate experiments were carried out. They differed only in that the experimental chamber in the second test was sprayed with DDVP emulsion at the rate of approximately 195 mg. of toxicant per square foot. These tests will be designated as Trial 1 and Trial 2, respectively.

In both instances the experimental animals were confined in the chamber for a two-week period. Groups of 10 male and 10 female weanling white rats were placed in each chamber in each trial. Five rats were housed in each cage. One rhesus monkey was placed in each chamber during Trial 1; two monkeys per chamber were used for Trial 2. All animals were observed daily for the development of any signs suggestive of DDVP intoxication. Body weights of all animals were taken three times weekly. Air samples were collected periodically from both experimental and control chambers during Trial 2 and analyzed for DDVP content by the method of total phosphorus.[3] A third chamber which had been sprayed with DDVP but in which no animals were housed was also tested for DDVP vapor concentration. Blood samples for cholinesterase determination were taken from half the rats and all the monkeys after one week and again after two weeks of confinement in the chamber. All the monkeys were also bled prior to any exposure and during the postexposure period until each poisoned animal regained its preexposure blood enzyme level.

Other tests involving respiratory exposure of rats were carried out in Hazleton-type chambers. In these tests six male and six female rats were confined per chamber, six animals of the same sex on each tier. The entire air supply available to the experimental groups was passed through a gas saturation bottle equipped with a fritted-glass disc and containing 250 ml. of DDVP. In some tests the total amount of DDVP in the gas saturation bottle was composed of two fractions, one of which had had previous aeration while the other had not. The rate of air flow was set at 160 cu. ft. per day. The animals remained in the chamber approximately 23 hours per day. They were removed from the chamber during the remaining hour and were allowed free access to

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food and water. Food, but no water, was also placed in the chambers. The control animals were similarly treated, except that no DDVP was put in the gas saturation bottles. Six separate trials involving minor variations in exposure conditions were carried out.

Each chicken tested was predosed orally with an aqueous solution of atropine sulfate at the rate of 15 mg/kg. Fifteen minutes later the designated dose of DDVP in peanut oil was administered subcutaneously.

All in vivo cholinesterase determinations were carried out by the electrometric method of Michel.[4] For those tests involving rats, 0.04 ml. of erythrocyte was used for each determination instead of the usual 0.02 ml. volume. Tests for determination of in vitro inhibition of cholinesterase were carried out by the method of Giang and Hall.[5] The enzyme used was purified bovine red cell cholinesterase.*

Results

1. *Acute Toxicity to the Rat.* –The acute dermal and oral L. D.$_{50}$ values for technical DDVP to adult male and female white rats are shown in Table 1. The corresponding values for parathion and for malathion, as determined under essentially identical conditions, are also given for comparison. It can be seen that in each instance DDVP is much more toxic to rats than malathion but considerably less toxic than parathion.

All rats killed by single dermal doses of DDVP died within 20 minutes after dosage, except a male dosed at 110 mg/kg. which survived for 40 minutes and another male dosed at 125 mg/kg. which survived for 17 days. With the exception of the latter animal, all the surviving rats appeared to be completely recovered within 24 hours after dosage. No significant degree of weight loss was noted among the survivors. The symptoms of poisoning observed were bulging eyes, excessive lacrimation, sialorhea, generalized muscle fasciculations, and tremors. Some of the animals were observed having convulsions just prior to death.

Following single oral doses of DDVP all rats which were killed died within one hour. Symptoms of poisoning noted were similar to those observed following dermal application. All the survivors appeared to be completely recovered within 24 hours after dosage.

It was of interest to determine the comparative toxicity of pure and technical preparations of DDVP. The pure DDVP contained greater than 99% of the active ingredient, while the technical material contained 90% DDVP. For this purpose an acute oral L. D.$_{50}$ study with pure and technical DDVP was conducted with the use of adult female rats. The L. D.$_{50}$ values were found to be 68 mg/kg. (with 19/20 confidence limits of 59 to 79) and 80 mg/kg. (with 19/20 confidence limits of 71 to [illegible]0) for pure and technical DDVP, respectively. These results indicate that, under the conditions of the test, pure DDVP is slightly more toxic than the technical grade material. The oral L. D.$_{50}$ of technical DDVP for female rats, reported above, is somewhat at variance with that given in Table 1, which was determined at a different time.

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* Obtained from Bios Laboratories, New York.

TABLE 1.—*Acute Oral and Dermal L. D.$_{50}$ Values for DDVP, Parathion, and Malathion to Male and Female White Rats*

Route of Administration	Sex	DDVP*		Parathion		Malathion	
		L. D.$_{50}$, Mg./Kg.	19/20 Confidence Limits, Mg./Kg.	L. D.$_{50}$, Mg./Kg.	19/20 Confidence Limits, Mg./Kg.	L. D.$_{50}$, Mg./Kg.	19/20 Confiden Limits, Mg./Kg
Oral	Male	80	62-104	15.0	10.2-16.5	1,375	1,208-1,56
	Female	56	48-65	3.6	3.2-4.0	1,000	855-1,1
Dermal	Male	107	84-137	21.0	14.0-34.0	>4,400	....
	Female	75	59-96	6.8	4.9-9.5	>4,400	....

* Technical grade material prepared at the Technical Development Laboratories

2. *Toxicity of Repeated Oral Doses to the Rat.*—Seven groups of ten young female white rats were fed for 90 days on diets containing 0, 5, 20, 50, 200, 500, and 1000 ppm, respectively, of DDVP admixed into their normal diet of ground Purina laboratory chow. These dietary DDVP contents corresponded to daily toxicant dosages of 0, 0.4, 1.5, 3.5, 14.2, 35.7, and 69.9 mg/kg., respectively, based on the actual food intake of the test animals. No significant degree of food refusal associated with dietary content of DDVP was noted. No signs of poisoning were noted in any of these rats; nor was any diminution in body weight gain associated with DDVP dosage seen.

Two rats from each group were bled for cholinesterase determination on the 3d, 11th, 60th, and 90th dosage days. The plasma cholinesterase values for these animals are shown in Figure 1. The rats fed at the 5 and 20 ppm levels showed an early transitory decrease in plasma enzyme activity, but by the 30th exposure day they were within the normal range. The 50 ppm group showed subnormal plasma enzyme activity through 60 days of exposure to DDVP, but, when tested on the 90th day, their values were normal. The animals fed 200 ppm showed a decreased plasma cholinesterase content through the 30th experiment day, but after this time an upward trend was noted, and by the 90th day their plasma enzyme level had returned to approximately normal. Those animals fed 500 and 1000 ppm, however, retained a low plasma cholinesterase level for the duration of the test.

The erythrocyte cholinesterase levels of these rats are shown in Figure 2. It may be seen from this Figure that the red cell enzyme values for the 5 and 20 ppm groups were indistinguishable from those for the controls. The 50 ppm group showed some early diminution of activity but was normal on the 30th day. However, the 200, 500, and 1000 ppm groups showed a progressively greater red cell enzyme diminution with increased DDVP content of the diet. This graded response to dietary toxicant remained evident throughout the test.

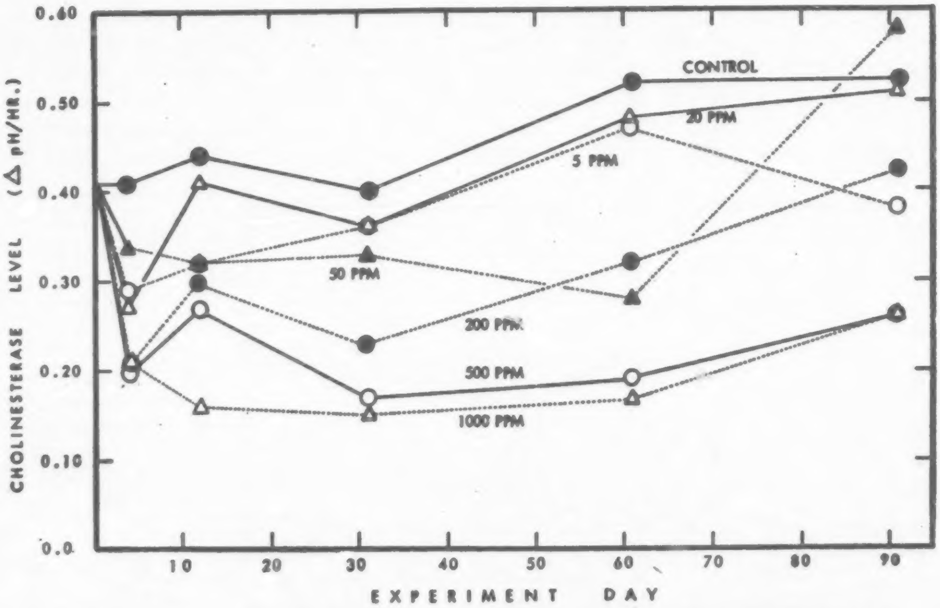

Fig. 1.—Plasma cholinesterase levels for groups of rats fed on diets containing DDVP. Each point represents the average value for two animals.

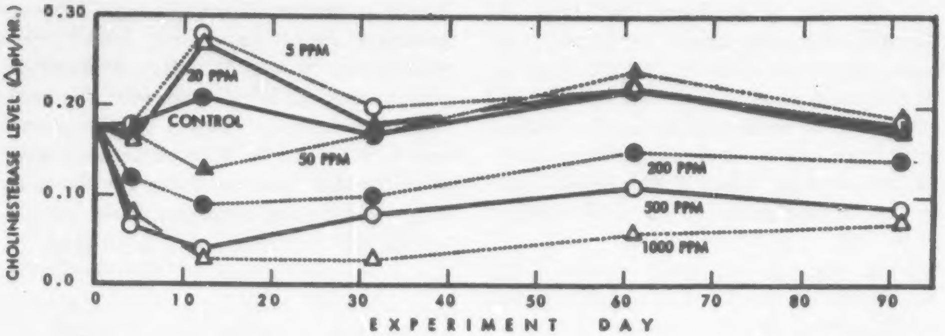

Fig. 2.—Erythrocyte cholinesterase levels for groups of rats fed on diets containing DDVP. Each point represents the average value for two animals.

3. *Toxicity of Repeated Dermal Doses to the Monkey.*—Three cynomolgus monkeys were given daily dermal doses of DDVP in xylene at the respective rates of 50, 75, and 100 mg/kg. This dosage was continued five days a week until the animals died.

Cholinergic signs appeared in all these monkeys, beginning in 10 to 20 minutes after administration of the first dose of toxicant. The signs noted in approximate order of their appearance included nervousness, gritting of the teeth, incoordination, muscle fasciculation, excessive salivation, labored breathing, miosis, and eventually inability (or indisposition) to move. The monkeys lay on the bottom of the cage and refused to move even when prodded. There was good correlation of rapidity of onset and severity and duration of signs with dosage level. At any given level the cholinergic effects tended to become severer with subsequent doses.

Blood samples were taken at intervals from all three monkeys for analysis of cholinesterase content. The results of these analyses for the monkey dosed at the intermediate level (75 mg/kg.) are shown in Figure 3. On the morning of the third day, after the monkey had received two doses of DDVP, the erythrocyte enzyme level was considerably depressed. With two subsequent doses of toxicant, the red cell enzyme activity fell somewhat lower and then maintained this low level even with continued dosage. The plasma cholinesterase content was found to be severely de-

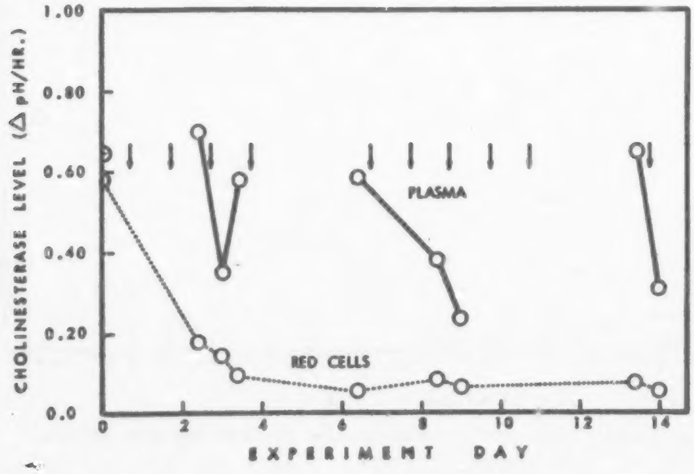

Fig. 3.—Plasma and red cell cholinesterase levels of a monkey given 10 daily dermal doses of DDVP at the rate of 75 mg/kg/day.

pressed soon after dosing but recovered rapidly, returning almost to normal overnight.

The cholinesterase response patterns were qualitatively similar at the other dosage levels and are not illustrated. The monkeys dosed at the 50, 75, and 100 mg/kg. levels died after receiving 8, 10, and 4 doses, respectively, of DDVP.

4. *Respiratory Toxicity.*—The concentrations of DDVP found in air from the Peet-Grady exposure chamber are shown in Figure 4. The DDVP concentration soon after spraying reached a peak value of about 6γ per liter. A rather rapid decrease then occurred, so that on the third day after the single application of spray the DDVP level was less than 1γ per liter. A rather low level of DDVP vapor (about 0.1γ to 0.2γ per liter) was maintained for the remainder of the two-week period. It should be borne in mind that, in addition to the respiratory exposure to DDVP, an unknown amount of dermal and oral exposure may have occurred as a result of condensation of DDVP vapor on surfaces.

No signs indicative of organic phosphorus poisoning were seen in any of the exposed animals at any time. Body-weight-gain curves for the rats were similar for the experimental and the control groups. No significant variation in weight was noted in the monkeys.

Plasma and erythrocyte cholinesterase levels are given in Table 2 for the rats. Each figure given is an average of five determinations. It appears that at the end of the first week the red cell and plasma enzyme levels for both sexes may have been somewhat lower in the experimental rats than in the control animals. However, both experimental and control groups appeared to be identical in this respect at the end of the second week of the test. These state-

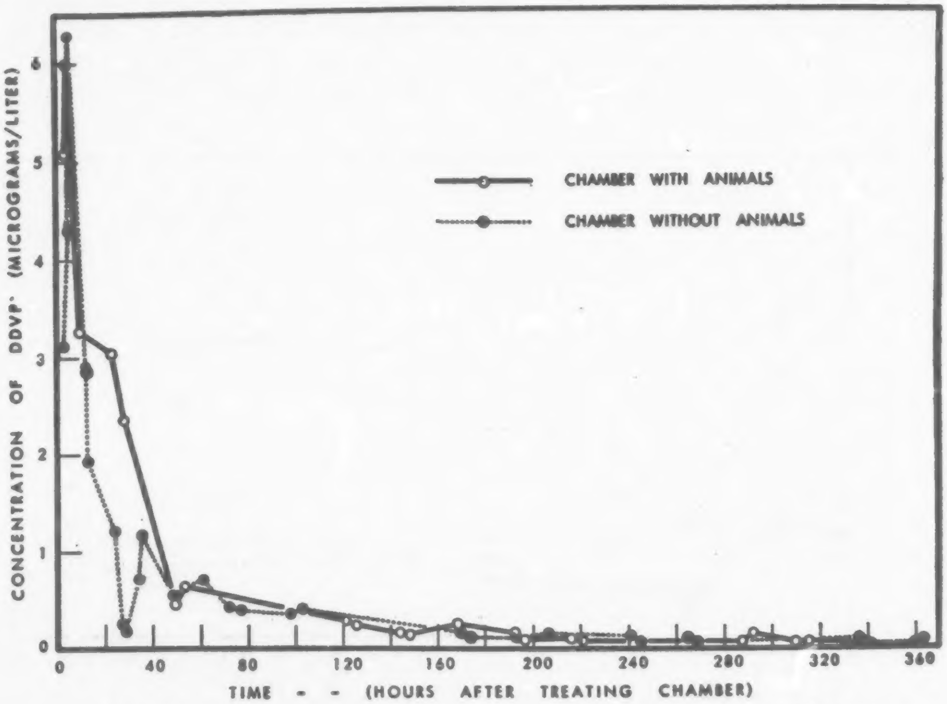

Fig. 4.—Vapor concentrations of DDVP in two chambers sprayed with DDVP-xylene emulsion.

TABLE 2.—*Plasma and Erythrocyte Cholinesterase Levels of Rats Exposed to DDVP Vapor and of Control Animals*

Concentrations of DDVP to Which These Animals Were Exposed Are Shown in Figure 4

Exposure Time	Sex	Toxicant	Cholinesterase Level, ΔpH/Hr.			
			Plasma		Erythrocyte	
			Trial 1	Trial 2	Trial 1	Trial 2
1 wk.	Male	DDVP Control	0.27±0.00* 0.30±0.02	0.29±0.01* 0.31±0.01	0.15±0.01* 0.18±0.00	0.22±0.01* 0.24±0.01
	Female	DDVP Control	0.27±0.01 0.30±0.01	0.32±0.01 0.33±0.01	0.17±0.01 0.21±0.01	0.21±0.01 0.21±0.01
2 wk.	Male	DDVP Control	0.33±0.01 0.33±0.01	0.33±0.01 0.32±0.01	0.17±0.01 0.21±0.00	0.17±0.01 0.16±0.01
	Female	DDVP Control	0.34±0.01 0.27±0.01	0.30±0.01 0.36±0.01	0.20±0.01 0.19±0.01	0.17±0.01 0.17±0.01

*Standard error of the mean

ments are based on the fact that, out of eight possible comparisons between the two groups, the experimental rats showed lower enzyme values seven times the first week. However, at the end of the second week the experimental group had lower values twice and higher levels three times out of eight comparisons. It should be stressed that in all cases the differences are small and of questionable significance.

The plasma and erythrocyte cholinesterase levels for the four monkeys in Trial 2 are shown in Figure 5. The results for the two monkeys in Trial 1 were similar to those for their counterparts in Trial 2. It is evident that at the end of the first week of exposure both the plasma and the red cell enzyme content of the experimental monkeys were much lower than their preexposure values. The plasma cholinesterase had recovered to some extent during the second week, but little change occurred in the erythrocyte esterase content. After cessation of exposure the cholinesterase con-

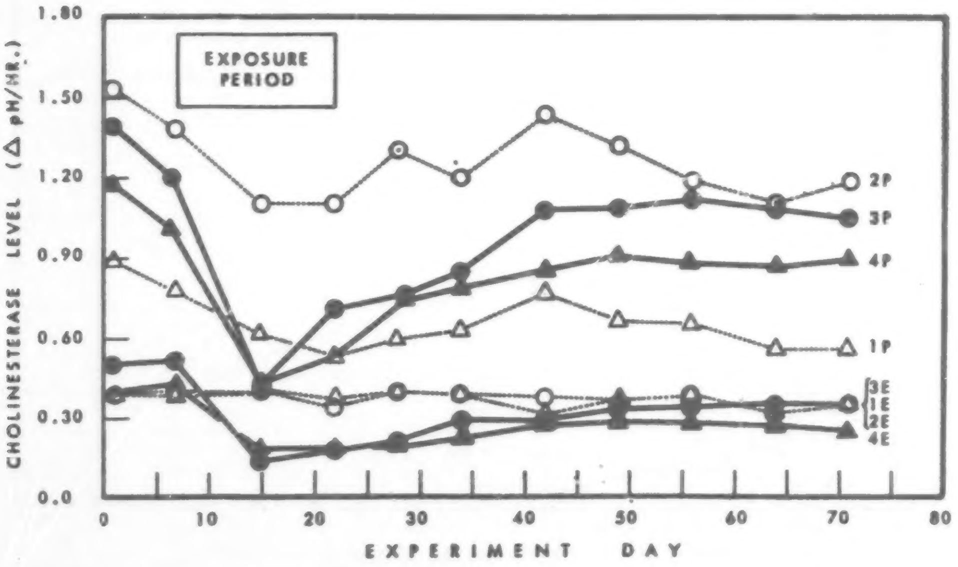

Fig. 5.—Plasma (*P*) and erythrocyte (*E*) cholinesterase level of controls (Monkeys 1 and 2) and of monkeys exposed to DDVP vapors (Monkeys 3 and 4) for indicated period.

tent of both blood moieties began to climb toward preexposure levels, the plasma returning rapidly and the red cells more slowly. There appears to be some indication of a downward trend in the plasma cholinesterase levels of the control monkeys. This may indicate that a small amount of DDVP vapor was present in the adjacent room and contaminated the control chamber during the normal process of air interchange. The red cell enzyme level for these control animals did not change significantly during the test.

In the case of both the rats and the monkeys the trend toward cholinesterase recovery apparent in the second week of exposure correlates well with the low concentrations of DDVP vapor found in the chamber at this time. The decreased cholinesterase activity noted at the end of the first week of exposure may very well have been due to the initial high concentration of DDVP in the chamber.

The experiments involving exposure of rats in the Hazelton-type chambers represented a much more rigorous test of the respiratory toxicity of DDVP. In these chambers it was easily possible to obtain lethal concentrations of DDVP in air. The results of these tests are summarized in Table 3. In Trial 3 determinations were made of the DDVP concentration in the air under various conditions. The results obtained from eight samples, taken just before exposure was begun, during a brief cleaning period, and after exposure was completed, showed an average DDVP concentration of 306γ per liter (range: 230 to 341) in the incoming air of the chamber. The exhaust air had an apparent average DDVP concentration of 17γ per liter (range: 14 to 23) based on 8 samples taken before the rats were placed in the chamber and 60γ per liter (range: 31 to 118) based on 12 samples taken after the animals were in the chamber. The significantly higher values noted after the animals were placed in the chamber are thought to be at least partly real and attributable to an increase in temperature inside the chamber because of the body heat of the animals. However, an apparent increase due to the phosphorus content of possible vaporized metabolic animal products cannot be ruled out.

The various trials with Hazleton chambers involved variations in previous saturation of the chamber and in preexperimental aeration of the DDVP. It may be seen from Table 3 that, as expected, previous saturation of the exposure chamber with DDVP vapor decreases the time required for the toxicant to affect the rats. However, an additional factor seems to be the preexperimental aeration of the DDVP. The decreased toxicity so induced may be due to (1) hydrolysis of DDVP by air-borne water vapor, (2) the rapid differential volatilization of a more toxic contaminant present in the supposedly pure material, (3) conversion of DDVP to a less toxic compound by oxidation, and/or (4) other unknown processes.

The initial signs of poisoning included slow labored respiration, sialorrhea, and paleness of the ears and feet. Under the severest conditions of exposure (Trial 4), these signs appeared within 2 hours and

Table 3.—*Exposure Conditions, Mortality, and Survival Time of Rats Exposed to DDVP Vapor in the Hazleton Chamber and of Controls*

Trial, No.	Toxicant	Preexposure Saturation of Chamber, Hr.	Preexposure Aeration of DDVP Sample, Hr.		Mortality (Died/Tested)		Average Survival Time, Hr.	
			200 Ml. Fraction	50 Ml. Fraction	Males	Females	Male	Females
1	DDVP	0	85	43	6/6	6/6	61.9	46.7
2	DDVP	0	0	0	6/6	6/6	26.4	25.8
3	DDVP	22	22	22	6/6	6/6	20.3	13.7
4	DDVP	41	41	0	6/6	6/6	6.9	10.1
5	DDVP	[illegible]	60	19	6/6	6/6	15.7	18.2
6	DDVP	147	[illegible]	118	6/6	6/6	52.3	43.0
7	Control	--	--	--	0/6	0/6	--	--

TABLE 4.—*Plasma and Erythrocyte Cholinesterase Levels of a Group of Laboratory Workers Exposed Daily in the Course of Their Duties to Rather High Concentrations of DDVP Vapor*

Subject, No.	Cholinesterase Level			
	Plasma		Erythrocyte	
	Preexposure	Exposure	Preexposure	Exposure
1*	1.00	1.03	0.68	0.66
2	0.85	0.78	0.70	0.50
3	0.81	0.85	0.84	0.60
4	0.75	0.68	0.75	0.51
5	1.00	1.06	0.77	0.54
6†	1.08	1.06	0.75	0.68

* Exposed less than other workers
† Not exposed

death occurred in male rats after 4.5 to 9.5 hours and in female rats after 4.8 to 17.5 hours.

A further, but less rigidly controlled, test of DDVP toxicity was afforded by the study of laboratory personnel working with the compound. Their exposure was largely respiratory, although occasional dermal contamination could not be ruled out and, in fact, was known to have occurred in at least one person. Five persons were constantly working with the toxicant during the seven-week period of the test. A sixth person who was not exposed to appreciable quantities of insecticides during this period was included as a control.

No illness attributed to the toxicant was noted by any of the subjects. However, some of the workers complained of a lingering, unpleasant, metallic aftertaste when working with technical DDVP or its concentrated solutions. The erythrocyte and plasma cholinesterase levels of all these men are given in Table 4. It is evident that four of the five men who were exposed showed moderate decreases in erythrocyte enzyme level. No significant variations in plasma cholinesterase levels were noted.

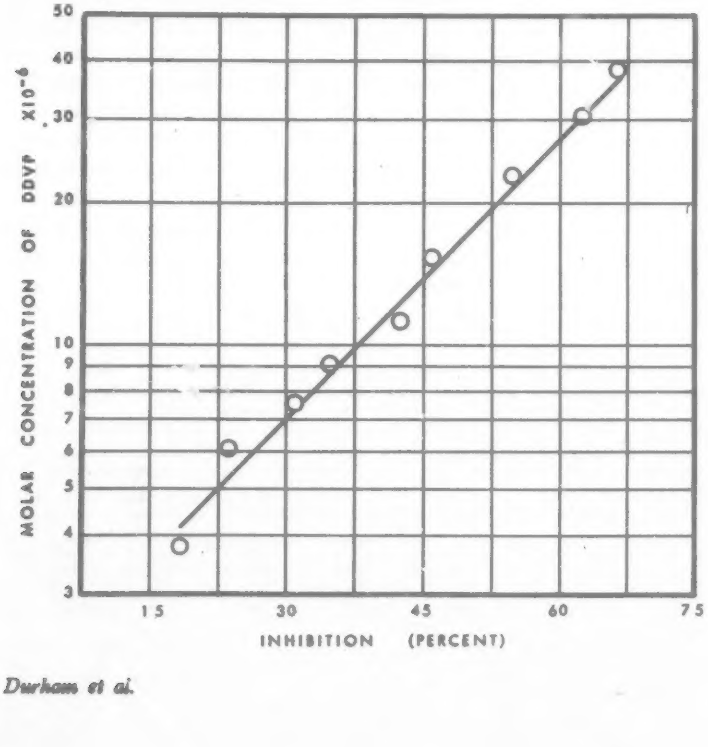

Fig. 6.—Inhibition of purified cholinesterase by DDVP in vitro.

5. *Screening Test for Neurotoxic Effect.*—In order to screen the compound for a possible neurotoxic effect, a series of 10 atropinized chickens was treated subcutaneously, 2 at each dosage level, with geometrically increasing dosages of DDVP beginning at 10 mg/kg. On the basis of the results it was estimated that the L. D_{50} of DDVP to atropinized hens by this route lay between 10 and 40 mg/kg. Eight additional hens were dosed in the same manner with 15 mg/kg. of DDVP.

The signs of poisoning seen in these hens were the cholinergic manifestations commonly associated with organophosphorus intoxication. No signs of paralysis or muscle weakness were noted. This test has been reported more completely elsewhere.[6]

6. *In Vitro Cholinesterase Inhibition.*—DDVP was found to be a potent inhibitor of cholinesterase in vitro as well as in vivo. Figure 6 shows the relationship which exists between DDVP concentration and enzyme inhibition. The concentration of DDVP necessary to cause 50% inhibition was determined to be 1.8×10^{-6} M, and the pI_{50} was calculated to be 5.66. No studies were carried out to investigate possible in vivo conversion of DDVP to a more powerful cholinesterase inhibitor, such as occurs with parathion, for example. However, comparison of our values for the toxicity and pI_{50} of DDVP with the figures given for other "nonactivated" inhibitors by Holmstedt[7] and Casida[8] indicates that DDVP has a toxicity in the expected range to be consistent with its in vitro cholinesterase inhibitory effect.

Summary

The acute oral and dermal L. D_{50} values of DDVP for male white rats were found to be 80 and 107 mg/kg., respectively. The corresponding values for females were 56 and 75 mg/kg. Pure DDVP was found, under the conditions studied, to be somewhat more toxic than the technical material.

Feeding for 90 days on dietary levels of DDVP up to 1000 ppm caused no signs of intoxication in rats. However, dietary levels as low as 50 ppm produced definite lowering of plasma and erythrocyte cholinesterase levels.

Three monkeys were killed by 8, 10, and 4 doses, respectively, of DDVP given dermally at the respective rates of 50, 75, and 100 mg/kg.

Monkeys confined for two weeks in Peet-Grady chambers which had been sprayed with 100 and 195 mg. of DDVP per square foot showed depletion of both plasma and erythrocyte cholinesterase. The concentration of DDVP in the air of the chambers sprayed at the rate of 195 mg/sq. ft. varied from about 6γ per liter immediately after spraying to 0.1γ per liter at the end of two weeks. Rats under the same condition showed a less definite enzyme inhibition. Neither species showed any clinical signs of organophosphorus intoxication.

Confinement in a Hazleton-type respiratory chamber, in which their entire air supply was essentially saturated with DDVP, proved fatal to rats in 4.8 to 83.0 hours, depending on conditions. The concentration of DDVP vapor exceeded 30γ per liter.

Some laboratory personnel constantly working with DDVP experienced an unpleasant metallic aftertaste as a result of their exposure. The four most severely exposed workers showed a small but definite decrease in erythrocyte cholinesterase level.

A screening test using chickens gave no indication of a neurotoxic effect resulting from severe DDVP exposure.

DDVP was found to be a potent cholinesterase inhibitor in vitro.

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